Effect of antidepressant treatment on social behaviour and circadian rhythms of locomotor activity in the rat

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EFFECT OF ANTIDEPRESSANT TREATMENT ON SOCIAL BEHAVIOUR AND CIRCADIAN RHYTHMS OF LOCOMOTOR ACTIVITY IN THE RAT

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To Dad

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CONTENTS

SUMMARY

1 The present study was based on the hypothesis that antidepressant efficacy is related to an ability to modify patterns of social behaviour or free-running circadian locomotor activity rhythms in rats.

2 Acute treatment of short-term isolated resident rats with non-sedative doses of the antidepressants clomipramine, fluoxetine, iprindole, mianserin and phenelzine, dose-relatedly reduced aggressive behaviour directed towards unknown intruder rats during social interaction (SI). Similarly, acute haloperidol (antipsychotic) or diazepam (anxiolytic) treatment dose-relatedly reduced aggressive behaviour, but these effects were associated with overt sedation. Conversely, only resident rats treated chronically with the antidepressants exhibited increased aggressive behaviour during SI which returned to the pre-treatment level by 7 or 14 (phenelzine-treated only; an effect thought to be related to the recovery of central monoamine oxidase activity) days post-treatment.

3 In rats housed in triads, subdominant members treated chronically with clomipramine or mianserin, at doses which increased aggressive behaviour during SI, exhibited an increased hierarchical position at the expense of the level of dominance exhibited by non-treated dominant rats.

4 Chronic treatment with clomipramine, fluoxetine or mianserin, at clinically-equivalent doses, had no effect on the phase-position of the free-running circadian locomotor activity rhythm expressed

by single or grouped rats. Conversely, clomipramine and fluoxetine, but not mianserin, disrupted circadian locomotor rhythmicity from 7 and 20 days of drug treatment, respectively.

In conclusion, it is argued that the diametrically different effects of acute and chronic antidepressant treatment on rodent social behaviour are indicative of reduced and increased social drive respectively, which, in the rat, is manifest by appropriate changes in aggressive behaviour exhibited during SI. It is further argued that the increase in social drive following chronic antidepressant treatment predicts antidepressant efficacy, while an ability to modify the free-running circadian locomotor activity rhythms of rodents is neither a predictor nor necessary characteristic of antidepressant efficacy.

CHAPTER 1 CLINICAL AND PHARMACOLOGICAL PERSPECTIVES OF AFFECTIVE DISORDERS - DEPRESSION

CHAPTER 1 CLINICAL AND PHARMACOLOGICAL PERSPECTIVES OF AFFECTIVE DISORDERS - DEPRESSION

1.1 Introduction

The objective of this chapter is to provide the reader with a concise introduction to the affective disorders, principally depression. Initially the clinical aspects of depressive illness will be described. The biochemical findings implicating abnormalities in the central serotonin and noradrenaline neurotransmitter systems will then be reviewed, followed by a description of the pharmacological treatment regimes available to the clinician. A review of the evidence implicating the involvement of circadian rhythm abnormalities in depression will be provided, followed by an appraisal of the current theories on the aetiology of depression. Finally, the methods currently employed to identify potential antidepressants will be appraised.

1.2 The Affective Disorders - Depression

1.2.1 Definition

The affective disorders are those conditions in which the main feature is an alteration of mood, or affect, to such a degree as to cause distress or to disrupt normal life. In these conditions mood may be abnormally elevated as in mania, or lowered as in depression. In addition, the central feature of the anxiety states is also a change in mood and as such are also included under the rubric 'Affective Disorders' (Hamilton, 1979a).

Most people experience fluctuations in mood between the acceptable limits of the happy-sad continuum. Where the self-assessment of mood tends to sadness then the individual may complain of feeling

depressed. Generally the experience is transient with no sequelae and as such is a normal, sometimes appropriate, emotion. However, the degree of depression may become so severe as to be clinically regarded as an illness causing severe distress, disrupting normal daily living patterns and being potentially fatal through attempted suicide, the ultimate expression of the disease state. It is therefore important not to confuse depressive illness, henceforth denoted by the term "depression", and depressed mood. It is the former aspect of depression, i.e. depressive illness, which attracts the attention of the clinician and provides the central theme of this thesis.

1.2.2 Classification

Much controversy has surrounded the classification of depressive illness. It is now recognized that the most important distinction between the different types of depression is that between unipolar, where patients show recurrent attacks of depression only, and bipolar depression, where patients exhibit fluctuations between episodes of depression and mania. The course of the two disorders can be distinguished by different characteristics. Angst (1981) observed that bipolar depression exhibited a more malignant course illustrated by earlier age of onset, higher frequency and total number of episodes during life with a shorter cycle length. In addition, bipolar depressives exhibited a lower rate of spontaneous remission with the consequence that bipolar psychoses had a longer duration than unipolar depressive disorders. Futhermore, patients with bipolar depression had a higher risk of developing severe organic brain syndrome. Surprisingly, however, the suicide rate was lower in the group of bipolar patients. Conversely, unipolar

depression exhibited a better prognosis illustrated by later age of onset with a lower number of episodes and greater time periods between episodes. While the duration of each episode was longer the total duration of the illness was shorter with such patients showing fuller remissions. Generally unipolar patients exhibited higher suicide rates than bipolar patients. Bipolar depression may be genetically distinguishable from unipolar depression (Angst and Scharfetter, 1979; Perris, 1976, cited by Katz and Hirshfield, 1978).

Within the group of depressions it is generally recognised that there are a number of subgroups; depression, therefore, would not appear to be a unitary condition. The main division advocated by Hamilton (1979a) has been into two types according to the clinical manifestations. The first is characterised by a retarded pattern of symptoms and has been described as vital, physiological or, more commonly, endogenous depression. In this situation the origins of the illness are largely unknown but once precipitated the illness continues on its own course. The second type may best be described as anxious depression, implying that various symptoms of anxiety dominate the profile of the condition. The syndrome has also been termed reactive or exogenous depression, implying that the condition occurs in reponse to an identifiable event. These two sub-divisions of depression have also been termed psychotic and neurotic depression respectively. Psychotic depression implies a more severe and incapacitating illness, most likely of organic origin, characterised by delusions, loss of insight and even hallucinations, whereas neurotic depression is milder and assumed to arise from neurotic conflict. The psychotic-neurotic distinction may be

considered to represent not two separate sub-groups of depressive illness but rather opposing ends of a continuum (Paykel et al., 1971), such that neurotic depression may mean simply the absence of any or all of the major characteristics of psychosis, i.e delusions and hallucinations, (Katz and Hirschfeld, 1978). A small group of depressed patients may be distinguished by the presence of hypochondriacal symptomology. Whether such patients indicate a true, independent sub-group is not clear since such patients may exhibit retarded behavioural patterns, i.e. endogenous depression, or agitation-anxiety, i.e. anxious depression, (Hamilton, 1979a). Another sub-group is termed involutional depression or involutional melancholia and is described as a syndrome which occurs with late onset (usually between 50 to 60 years of age) in individuals with a rigid obsessional personality. It is characterised by anxiety, agitation, hypochondriasis and sometimes paranoia. By their nature the terms used to identify possible sub-groups of depression introduce aetiological and symptomatic considerations that are not always accurate or justified. Indeed, whether the pattern of symptoms distinguishing involutional depression truly identifies a separate syndrome is a topic of much debate (Hamilton, 1979a).

The affective disorders may also be sub-divided according to the primary-secondary distinction introduced at the Williamsburg Conference in 1969 by Robins and Gruze (cited by Katz and Hirschfeld, 1978). The primary affective disorders were defined as depressive (or manic) episodes occurring in patients whose previous histories were free of psychiatric disturbance or contained previous episodes of depression or mania but no other psychiatric illnesses. Secondary affective disorders were defined as those occurring in patients with

a pre-existing, diagnosable psychiatric illness other than primary affective disorder. This nosological distinction was proposed primarily for research (rather than clinical) purposes, and indeed primary and secondary depressives may be clinically indistinguishable. Even so, the distinction predicts that primary depressive patients will have more affective psychopathology in their first degree relatives, while the psychopathology of patients with secondary depression will resemble those of other patients with their underlying condition rather than those with primary depression.

1.2.3 Epidemiology of Depression

The prevalence of depressive illness has been extensively studied both in terms of incidence in the general population and the number of people interviewed by general practitioners and psychiatrists. Such studies have indicated an annual rate for depression of approximately 0.35% for a given population, the most common social group being women aged between 20-40 years, of whom only 10-15% warrant hospitalisation (quoted by Green and Costain, 1981). The vast majority of patients thus receive treatment while being fully integrated into normal society, and are therefore subjected to the normal pressures of everyday life throughout the period of their depressed state. Successful treatment of the depressed patient must therefore result in a change in that patient's response to environmental and social stimulation, which prior to treatment the patient would have found intolerable.

1.2.4 Symptomology and Diagnosis of Depression

1.2.4.1 Symptomology

The principal features of depression, i.e depressed mood, feelings of guilt and suicidal tendencies, have been described in depth by Hamilton (1979a), from which the following description of depressive illness has been obtained. In the mild stages of the illness the principal features of depression are not obvious and the patients usually complain of lack of energy and tiredness, low mood (which frequently fluctuates throughout the day and generally improves as the day proceeds), difficulties with sleep, and loss of interest, appetite and libido or sexual drive resulting in impotence or frigidity. Related to the loss of interest is an increased difficulty in coping with work, which the patient tends to avoid, associated with indecisiveness and lack of self-confidence. Sleep disturbance increases such that patients display early wakening, interrupted sleep, difficulties in falling asleep and unpleasant dreams. The increased loss of appetite subsequently results in weight loss. As the severity of the illness increases the depressed mood becomes constant and unresponsive to stimuli which normally elevate mood. The patient feels isolated and tends to avoid company. Patients become self-reproachful and blame themselves for their failure to overcome their depression and their presumed neglect of their family such that they become increasingly preoccupied with their deficiencies and feelings of guilt. Suicidal tendencies begin with the increased feeling that life is not worth living and thoughts of death become a preoccupation.

Symptoms of anxiety are common in depressives. The patients become anxious, tense, irritable, forgetful and ill-tempered, have

difficulty in concentrating and worry about trivial matters. The somatic manifestations of anxiety (i.e. headaches, sweating, palpitations, trembling, indigestion and flatulence) may become prominent and overlap with the physiological symptoms. In the more severe stages of the illness the pupils become dilated and the hands tremor.

Modified psychomotor performance (i.e. retardation or agitation) is not always present during the early stages of the illness. In the milder stages of depression both retardation and agitation may be present but as the illness progresses one eventually tends to mask the other. Initially retarded depressives tend to walk slowly or sit motionlessly, not rigidly but with reduced body movements and poverty of facial expression, reflecting a deficiency of alertness and increased unresponsiveness to environmental stimuli. In the early stages speech is low and monotonous but not slowed, even though there may be a delay in responding to questioning. This does not necessarily reflect a slowness of thinking but rather a preoccupation with continuous miserable thoughts. As the illness progresses the retarded depressives show increasing periods of sitting motionless with a rigid posture. Both speech and thoughts become slowed such that eventually the patient becomes mute and unresponsive to questioning. In the most severe stages it becomes increasingly difficult to persuade the patient to eat or drink, sleep becomes grossly disturbed, and the fixed miserable facial expression completes the isolation from human contact. At this stage the patients may express, if they can be persuaded to talk, delusions of guilt and experience of hallucinations. Agitation first appears as restlessness involving pacing and fidgeting. As the

agitation increases the patients fidget constantly, are unable to sit still and pace restlessly. In the more severe stages of agitation patients continually wring their hands, pull at their clothes, hair or faces and moan. The severe symptoms are now very rarely observed since the vast majority of patients respond well to antidepressant treatment (Hamilton, 1979a; Green and Costain, 1981).

Conversely, the symptoms of mania are characterised by an abnormal elevation of mood. During the initial stages of the illness the patient exhibits increased activity, optimism and cheerfulness. The patient becomes excessively friendly, sexually and socially uninhibited and tactless, is occupied with many different tasks, schemes and ideas, and talks continuously. Although mood is highly elevated the patient cannot tolerate criticism or advice and can quickly become irritable and quick to violent anger. In work the patient makes overestimated judgements and makes decisions that cannot be met either financially or practically. It is typical that the patient shows little insight and when challenged insists that he/she has never felt better and asserts that his/her thinking and ideas are clearer than ever before. As the illness progresses so sleep requirements diminish and appetite and libido increase. The patient eventually exhibits ceaseless activity and talking, euphoria and rage; hallucinations and delusions may also be present (Hamilton, 1979a; Green and Costain, 1981).

1.2.4.2 Diagnosis and Rating Scales

Only the persistence of the initial symptoms of depression, to the point where normal life becomes difficult, prompts the patient to

seek consultation with a general practitioner. If depressive illness is suspected then the patient is referred to a consultant psychiatrist. The clinical features of the early stages of depression are clearly of primary importance for diagnosis, classification and management. In order to standardize the assessment and evaluation of the described symptoms a number of rating scales have been developed. The types of rating scales used fall into two broad groups depending on whether the rating is based on clinical interview (observer scales) or solely by the patient (self-assessment scales).

The most widely used observer rating scale is that produced by Hamilton (1960), sometimes referred to as the Hamilton Rating Scale (HRS) or Hamilton Diagnostic Scale (HDS). Ratings are based on clinical interview and the scale designed to cover the previous week. The scale contains 17 variables, including both mental and physical symptoms, which are scored and the values summed to produce an overall rating of the severity of the illness. A major criticism of the HRS is that due to its design ratings cannot be obtained more frequently than at weekly intervals. Thus the system cannot identify specific short-term changes in the symptomology of depression. Over the long-term, however, the HRS has proved to be a useful indicator not only of the time-course of depression and its severity but also of the general remission from depression during treatment.

Self-assessment scales are of most value when assessments have to be made frequently and there are a number in current use. The Beck Depression Inventory (BDI; Beck et al., 1961) appears to be the most popular and, for self-assessment purposes, consists of 13 items to be graded by the patient (see also Hamilton, 1979b). Other

examples of self-assessment scales are the Zung Self-Assessment Depression Scale, the Lubin Adjective Check List, the Popoff Index of Depression and the Wakefield Scale (see Hamilton, 1979b, and references cited therein). The scales differ in the number of items used and their validity has been examined by determining the correlation to the HRS. In some cases this has resulted in a refinement of the scale to increase its correlation to the HRS such that whether the scales provide additional information to the HRS is open to question. In addition it is impossible for patients to assess the incidence or severity of certain symptoms, e.g. loss of insight and delusions, and retardation, agitation and hypochondriasis, respectively, and such self-assessment scales are also open to the biases of the patient. Generally, therefore, observer ratings, such as the HRS, appear to be more valid and provide a clearer overall appreciation of the symptomology of depression than the self-assessment scales. The classification into diagnostic categories is aided by the International Classification of Diseases system and the Diagnostic and Statistical Manual (see Gelder et al., 1983).

Other rating scales may be employed to examine specific symptoms of depression. For example, the Hostility and Direction of Hostility Questionnaire (HDHQ) attempts to quantify the psychological problems associated with a failure of interpersonal relationships and exaggerated levels of aggression or hostility (see Priest et al., 1980, and references cited therein). The questionnaire consists of 51 items grouped into 5 scales measuring both extrapunitive (outward) hostility (i.e. the urge to act out hostility, criticism of others and projected hostility) and intropunitive (inward) hostility (i.e.

self-criticism and guilt). Hostility levels are high in depressive illness, especially those directed towards oneself (i.e. intropunitive scales). Both the total hostility and the degree of intropunitive hostility decrease as the patient recovers from the illness (Priest et al., 1980).

1.3 Neurotransmitter Abnormalities in Depression

1.3.1 Historical Perspectives

Three observations led to the development of the idea that alterations in central monoamine metabolism and function could precipitate a mood change and that such changes may be central to the pathogenesis of depressive illness.

During the early 1950's the anti-tuberculosis drugs isoniazid and, more especially, its isopropyl derivative, iproniazid, were observed to produce mood-elevating effects in man. Subsequently Zeller et al. (1952) showed that iproniazid, in contrast to isoniazid, was capable of inhibiting the enzyme monoamine oxidase (MAO), the major degradative enzyme of the monoamine transmitters serotonin, i.e. 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA) (Cooper et al., 1978).

Secondly, in 1954 Wooley and Shaw proposed that 5-HT might be involved in the regulation of mood (cited by Green and Costain, 1981). Their hypothesis was based on consideration of the hallucinogenic effects of lysergic acid diethylamide (LSD) and its structural similarity to 5-HT. In addition it was known that LSD was a 5-HT antagonist on smooth muscle <u>in vitro</u>.

The view that central monoamines were involved in mood regulation was further supported by the clinical observation that the alkaloid reserpine, which during the 1950's was used to treat hypertension, could precipitate a severe depressive episode clinically indistinguishable from endogenous depression. It was subsequently demonstrated that reserpine had the ability to deplete the central monoamines 5-HT (see Carlsson et al., 1957b, and references cited therein), NA and DA. In addition, Carlsson et al. (1957a) demonstrated that the reserpine-induced effects in animals could be reversed by 3,4-dihydroxyphenylalanine (L-DOPA), the precursor of DA and NA (Cooper et al., 1978), either alone or, more effectively, in combination with 5-hydroxytryptophan (5-HTP), the precursor of 5-HT (Cooper et al., 1978).

In the light of these observations numerous studies have attempted to identify biochemical changes that may occur in depressive illness. Two general approaches have been used. The first has been to examine for biochemical changes in tissues obtained from depressive patients such as blood, cerebrospinal fluid (CSF), urine, platelets and post-mortem brain tissue. This approach, with reference to serotonergic and noradrenergic neurotransmitter systems in the central nervous system (CNS), will be briefly reviewed here (section 1.3.2). The release of inhibitory and releasing factors from the hypothalamus (which control the release of hormones originating from the anterior lobe of the pituitary), and the pineal hormone melatonin, are ultimately under the control of central monoamine systems. Central monoamine activity may therefore be reflected in the release of anterior hypophyseal hormones or melatonin, and many studies have attempted to identify possible hormonal markers in

depressed patients that may be indicative of abnormal central monoamine activity. The findings of such studies on the secretion of adenohypophyseal hormones will be described briefly in section 1.3.3, while the control of melatonin release and abnormalities of melatonin secretion in depression will be described briefly in sections 1.6.2 and 1.6.3 respectively. The second approach is to examine the ways that drugs effective in treating depressive illness alter the biochemical function of the brain in experimental animals, or biochemical parameters of depressed patients, and will be reviewed in section 1.4.5.

It should be noted that changes in electrolyte distribution have been observed in some depressed patients. Electrolytes, such as sodium, potassium, calcium and magnesium, have profound biological importance regarding, for example, the resting potential of neurons and other excitable cells, propagation of action potentials, and consequently the release of neurotransmitters. For the sake of brevity these changes will not be reviewed here. Instead, the interested reader is directed to the review by Coppen (1967).

1.3.2 Neurotransmitter Abnormalities

1.3.2.1 The Serotonin System

The observation by Ashcroft et al. (1966) that 5-hydroxyindole compounds in the CSF of patients were lowered in depression sparked a variety of studies on the biochemical markers of the indoleamine system in depressed patients. This research area has been extensively reviewed by a number of authors (e.g. Ridges, 1976; Murphy et al., 1978; Green and Costain, 1979, 1981; Sugrue, 1981a; Dunner, 1983). Only a brief review will be provided here.

Asberg et al. (1976) reported that concentrations of 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of 5-HT in the CNS (Green, 1978; Fuller, 1985) in the CSF of depressed patients exhibited a bimodal distribution; one group having normal and the other lower 5-HIAA levels. The latter group showed an increased tendency to attempt suicide (see also Shaw et al., 1967). Furthermore, decreased levels of 5-HT and 5-HIAA have been detected in some, but not all, studies on post-mortem brain samples from suicides (see review by Murphy, 1978). Consequently, it has been suggested that low CSF 5-HIAA levels may be predictive in identifying individuals with a greater predisposition to commit suicide (Oreland et al., 1981; Agren, 1983; van Praag, 1986b), although this view has recently been questioned by Secunda et al. (1986) who found no correlation between CSF 5-HIAA levels and suicide attempts. Indeed, although suicide is assumed to be the ultimate expression of depression, whether all individuals who attempt suicide are also depressed is questionable. Van Praag and co-workers have utilized the ability of probenecid to inhibit the acid transport system in the brain, thereby blocking 5-HIAA egress from the CSF to the blood stream, to study 5-HT turnover in depression. A decreased 5-HIAA accumulation following probenecid treatment is assumed to suggest decreased 5-HT turnover. These studies (van Praag and de Haan, 1979; van Praag, 1979, 1986a, and references cited therein) indicated that 5-HIAA accumulation after probenecid treatment was invariably reduced in patients with endogenous depression. Furthermore, in half of the cases 5-HIAA accumulation returned to normal following recovery. Those patients who had apparently recovered from depression but still showed persistent low CSF 5-HIAA levels after probenecid, and their family members, exhibited

increased depression rates (van Praag, 1979). These observations suggest that disturbed 5-HT function predisposes the individual to depressive episodes rather than being a causal factor. A major problem with CSF 5-HIAA studies is determining the origin and significance of the 5-HIAA measured. Indeed, some studies indicate that the majority of CSF 5-HIAA may be spinal in origin (see Green and Costain, 1979); furthermore, whether central 5-HIAA levels reflect central 5-HT function is questionable since, in rodents at least, it appears that 5-HT is synthesized in excess of functional needs (Green and Grahame-Smith, 1975). The level of CSF 5-HIAA may, therefore, merely reflect changes in the intraneuronal metabolism by MAO (see also the behavioural studies of Wolf et al., 1985).

5-HT does not cross the blood-brain barrier from the peripheral circulation and must therefore by synthesized in the brain from its precursor L-tryptophan, an essential amino acid obtained from dietary protein (Cooper et al., 1978). Central 5-HT synthesis is, at least in part, dependent on the brain tryptophan level which, in turn, is dependent not only on the free plasma tryptophan concentration but, more importantly, on the level of the latter in relation to that of other large neutral circulating amino acids which compete for the same uptake process into the brain (Wurtman and Fernstrom, 1976; Mandell and Knapp, 1977; Green and Costain, 1981; Wurtman et al., 1981). Thus decreased plasma tryptophan levels would ultimately influence tryptophan availability for central 5-HT synthesis, and indeed some reports indicate that both free and total plasma tryptophan levels are reduced in depressives (Fiore et al., 1979; Moller et al., 1980), however, others have failed to confirm this view (Green and Costain, 1979, and references cited

therein; Dam et al., 1984). The majority of tryptophan is metabolized in the liver by tryptophan pyrrolase which may be induced by corticosteroids (Green and Costain, 1979). The reduced plasma tryptophan levels may therefore simply reflect increased peripheral tryptophan metabolism induced by high cortisol levels which have been observed in some depressed patients (see section 1.3.3 below). If a simple deficiency of tryptophan availability produced depression then lowered CSF tryptophan levels should be observed in depressives and 5-HT precursor administration (with tryptophan or 5-HTP) should be an extremely effective antidepressant. Some studies have indeed demonstrated lower CSF tryptophan levels in depressives; however this is not invariably so (see Green and Costain, 1979, and references cited therein). Moreover, while precursor treatments increase central 5-HIAA levels in depressed patients (indicating that the enzymatic processes involved in 5-HT synthesis are not impaired, but may only reflect increased intraneuronal 5-HT metabolism by MAO) only a sub-group of depressives respond to such treatment (van Praag, 1981b, and references cited therein).

Blood platelets are the only non-neuronal cells that have the ability to accumulate, store and metabolize substances which are either established or putative neurotransmitters, and this particularly applies to 5-HT. Furthermore, the processes involved in 5-HT uptake, storage and metabolism (by MAO type B) by platelets appears to be similar to that observed for 5-HT in the brain (see review by Boullin, 1978). Consequently the platelet has been advocated as a model for central 5-HT function; however this view is at best somewhat tenuous. Decreased platelet accumulation of 5-HT in depressed patients has been observed in some studies (Hallstrom et

al., 1976; Arora et al., 1984; Healy et al., 1986a, 1986b; Healy and Leonard, 1987) but not in others (Shaw et al., 1971, cited by Green and Costain, 1979). The discrepancy between these results may be due to platelet 5-HT uptake exhibiting a circadian rhythm in normals but not in depressed patients (Healy et al., 1986a, 1986b) which is restored on recovery. Furthermore, platelet 5-HT also shows marked seasonal variation in depressed patients (Arora et al., 1984; Egrise et al., 1986). Studies on platelet MAO activity have yielded contradictory results (see review by Rotman, 1983) with a number of studies showing little relationship between platelet MAO activity and the degree of depression (see also Honecker et al., 1981; Maubach et al., 1981).

Most 5-HIAA in the urine is thought to arise from the gut with only a small fraction originating from the CNS (see Green and Costain, 1979). Such measures, therefore, have limited value, if any, as markers of central 5-HT activity, and thus will not be discussed here.

The general consensus of opinion in the literature is that the data, especially that arising from CSF studies, indicate the possibility of lowered 5-HT concentration or metabolism in depression.

1.3.2.2 The Noradrenaline System

The functional status of central catecholaminergic systems in depression has been extensively investigated by measurement of metabolite levels in urine, plasma and , to a lesser extent, CSF. These areas have been extensively reviewed by a number of authors (e.g. Schildkraut and Kety, 1967; Schildkraut, 1978; Green and

Costain, 1979; Sugrue, 1981a; van Praag, 1981a; Dunner 1983). The findings of these investigations, with respect to the noradrenergic system, will only be summarized here.

The major metabolite of NA originating from the CNS is thought to be 3-methoxy-4-hydroxyphenylglycol (MHPG) (Cooper et al., 1978), or its sulphate ester conjugate, and thus changes in the level of MHPG in the urine, plasma or CSF are thought to reflect central noradrenergic activity. Studies of MHPG levels in depressed patients have generally yielded equivocal results. Thus, urinary or plasma levels of MHPG in patients with endogenous depression have been shown to be lower (Maas et al., 1974, cited by Ridges, 1976; Wehr et al., 1980; Agren, 1983; Puzynski et al., 1984; Secunda et al., 1986), normal (Oreland et al., 1981) or raised (Agren, 1983; Halaris, 1984) compared to normal controls (see also review articles cited above). Furthermore, Puzynski et al. (1984) observed that reduced urinary MHPG levels were observed in all types of affective illness without being related to the type of depressive syndrome, while the reduced levels of urinary, plasma or CSF MHPG levels observed by Agren (1983) or Secunda et al. (1986) appeared to identify potential suicides in a similar manner to reduced CSF 5-HIAA levels (see section 1.3.2.1). The studies of Schildkraut and co-workers (see Schildkraut, 1978, and references cited therein) suggest that MHPG concentrations in unipolar depressive patients are invariably higher than that observed in patients with bipolar depression. Furthermore, urinary MHPG levels of bipolar patients were relatively lower during periods of depressive and higher during manic or hypomanic episodes than during periods of remission. These observations would strongly argue against a homogeneous population of

depressive patients. Indeed, the level of MHPG excretion in unipolar patients has been reported to correlate with response to specific antidepressant drugs. Thus, patients with low urinary MHPG levels reportedly respond to drugs which selectively (albeit slightly) increase central noradrenergic activity (i.e. desipramine and imipramine), while patients with higher levels of MHPG respond to drugs which also increase central serotonergic activity (i.e. amitriptyline) (see Schildkraut, 1978, and Dunner, 1983, and references cited therein). It is claimed that these results indicate that low urinary MHPG levels and favourable response to imipramine or desipramine is indicative of decreased central noradrenergic activity, while high urinary MHPG levels and favourable response to amitriptyline is indicative of decreased central serotonergic activity; at best this view would appear somewhat tenuous. It should be noted at this point that the drugs indicated above inhibit the re-uptake of central NA and 5-HT with varying degrees of relative selectivity, which for these drugs is slight or, for amitriptyline, at best marginal; the mode of action of these antidepressants will be discussed in section 1.5.3.

The general consensus of opinion in the literature is that studies of MHPG levels indicate the possibility of abnormal noradrenergic activity or NA metabolism in patients with affective disorders.

1.3.3 Endocrine Abnormalities in Depression

A number of abnormalities in the release of hormones originating from the adenohypophysis have been observed in some depressed patients. Since the release of these hormones is ultimately controlled by central monoamine neurotransmitters such observations are indicative

of abnormalities in central monoamine function, and will only be briefly described here.

Some depressed patients exhibit a blunted growth hormone (GH) response to challenge with pharmacological agents which increase GH secretion (van Praag, 1981a; Siever et al., 1983; Meltzer et al., 1984). Furthermore, Garver et al. (1975, cited by van Praag, 1981a) demonstrated a correlation between human GH response to insulin and renal MHPG excretion. The urinary concentration of MHPG is regarded as a crude indicator of central NA metabolism (Cooper et al., 1978; Green and Costain, 1981), and thus the correlation indicates that the GH response to insulin diminishes as central NA turnover diminishes. These observations suggest that central noradrenergic systems may be deficient in depression.

Abnormalities in luteinizing hormone (LH) (van Praag, 1981a) and prolactin (Linkowski et al., 1980; see also Meltzer et al., 1984) levels in postmenopausal women with unipolar depression have been observed. These observations suggest that the noradrenergic system which ultimately controls LH release, and the inhibitory dopaminergic or stimulatory serotonergic systems controlling prolactin release may be abnormal in depression (see van Praag, 1981a).

A blunted thyroid-stimulating hormone (TSH) response to thyrotropin-releasing hormone (TRH) (Gold et al., 1980; Sternbach et al., 1985) or low 24h levels of serum TSH (Kjellman et al. (1984) have been observed in some depressed patients. These effects are thought to be secondary to decreased sensitivity of the pituitary

thyrotrophs following a prolonged increase in TRH stimulation (see van Praag, 1981a; Kjellman et al., 1984). In addition, there is increasing evidence that the thyroid hormones exert a considerable influence over the central function of monoamine neurotransmitters (e.g. see Atterwill, 1981; Vaccari, 1982; Heal et al., 1987). Furthermore, TRH has been used, with some success, to treat unipolar depression in women (Pecknold and Ban, 1977).

The adrenal cortex secretes cortisol under the influence of adrenal corticotrophic hormone (ACTH) from the pituitary, which is secreted in response to the hypothalamic corticotrophin releasing factor (CRF). Cortisol secretion is maintained predominantly by the levels of cortisol exerting a negative feedback on both ACTH and CRF; in addition there is evidence of a noradrenergic system with a tonic inhibitory effect on CRF production (see van Praag, 1981a). Some depressed patients exhibit abnormalities in the hypothalamus-pituitary-adrenal axis as indicated by the hypersecretion of cortisol (e.g. see van Praag, 1981a; Claustrat et al., 1984) and corticosterone (Arana et al., 1985). These observations suggest that in a subgroup of depressed patients there is a failure in the negative feedback system. Furthermore, normal subjects treated with dexamethasone (a synthetic steroid) at the nadir of the circadian rhythm of cortisol secretion (see Wetterberg et al., 1982) secrete virtually no cortisol the next day, while patients with vital depressions may exhibit resistance to the effect of dexamethasone (see Carroll, 1985). Resistance to the dexamethasone suppression test (DST) is thought to have some clinical value firstly, in differentiating some affective disorders (Arana et al., 1985; Baldessarini and Arana, 1985); secondly, in identifying depressed

patients with a high risk of attempted suicide (Robbins and Alessi (1985), although this ability has been questioned (Secunda et al., 1986); and lastly, in predicting the response to drug therapy (Amsterdam et al., 1983; Christensen et al., 1985), including the potential of early relapse (Greden et al., 1980), or specific somatic antidepressant treatments such as sleep deprevation (Nasrallah and Coryell, 1982). The use of the DST in psychiatry has been a topic of discussion since its inception and its diagnostic and predictive value is still controversal (see Carroll, 1985; Baldessarini and Arana, 1985; Schatzberg, 1985).

In conclusion therefore, sub-populations of depressed patients have been shown to exhibit hypersecretion of corticosteroids, while major sub-groups of depressed patients demonstrate a diminished GH response to insulin-hypoglycemia, a blunted TSH response to TRH and low levels of plasma LH. Hypersecretion of cortisol is also observed on hyperactivity of the adrenal cortex (Cushings syndrome) or when hypersecretion of ACTH from the pituitary (Cushings disease) induces overactivity of the adrenal cortex resulting in increased secretion of cortisol. In both cases the neuroendocrine abnormalities listed above are observed. Furthermore, evidence suggests that administration of exogenous corticosteroids may precipitate these same neuroendocrine abnormalities. These observations suggest that hypersecretion of corticosteroids may be the underlying cause of the other neuroendocrine changes observed in depressed patients (see Kendler and Davis, 1977). In support of this hypothesis, Horton et al. (1986) observed that depressed patients who failed to suppress plasma cortisol levels in the DST also exhibited a blunted GH response to the $alpha_2$ agonist, clonidine.

1.4 Treatment of Depression

1.4.1 Historical Aspects

The earliest of the current treatments for depression was electroconvulsive therapy (ECT), introduced in 1938 by Cerletti and Bini (cited by Green and Costain, 1981). The usefulness of ECT in depression became apparent with clinical experience, and, while it is not a pharmacological treatment, it is reasonable to assume that its effects are mediated via central neurotransmitter systems. ECT remains, however, the least understood and the least critically assessed of the antidepressant treatments. The major pharmacological advances in the treatment of depression arose with the advent of the monoamine oxidase inhibitors (MAOI's) and tricyclic antidepressants (TCA's) in the 1950's. The efficacy of these compounds is thought to be due to the resulting increase in the synaptic concentration of central 5-HT and/or NA following either inhibition of MAO, the major degradative enzyme of these neurotransmitters, or inhibition of the active re-uptake processes, respectively. More recently, a new generation of putative antidepressants have been developed which, although some are cyclic in structure, bear little structural similarity to the previously available antidepressant drugs. Some compounds have been developed as relatively specific re-uptake inhibitors (generally for 5-HT), while others are demonstrably effective clinically but whose mode of action cannot be attributed to inhibition of either MAO or neurotransmitter re-uptake. These latter compounds are generally known as the atypical antidepressants.

The following sections provide a brief introduction to the development and clinical use of the various classes of drugs currently available to the clinician, i.e. MAOI's, monoamine

re-uptake inhibitors (TCA's and the more recent "second generation" monoamine-specific re-uptake inhibitors) and the atypical antidepressants, together with a brief description of their pharmacological and biochemical effects following acute and chronic treatment. The use of ECT or lithium in depression, and the effect of equivalent treatments on various behavioural animal models or biochemical measures, will not be discussed here except where such effects are pertinent in comparison to a particular drug-induced effect. Instead, the interested reader is directed to the reviews of Green and Costain (1979, 1981), Crow and Johnstone (1979) and Shaw (1979).

1.4.2 Monoamine Oxidase Inhibitors

The observation of the mood-elevating effects of iproniazid in man, and the subsequent demonstration by Zeller et al. (1952) that iproniazid inhibited MAO activity, led to the development of the group of compounds known as the MAOI's. The historical development of the MAOI's for the treatment of depression has been reviewed by Kline and Cooper (1980).

1.4.2.1 Monoamine Oxidase

MAO is a flavoprotein associated with the outer mitochondrial membrane (Youdim and Findberg, 1983). It is widely distributed in almost all mammalian tissue, including the CNS, with varying degrees of activity. Only about 6-8% of brain MAO is located intraneuronally, where it is the most important enzyme involved in the metabolism of 5-HT and the catecholamines (Cooper et al., 1978; Green and Costain, 1981), with the majority of brain MAO located extraneuronally (Youdim and Findberg, 1983). Studies have indicated

that MAO is not a single entity but is composed of a number of closely related enzymes (Youdim and Findberg, 1983, and references cited therein). It is now established that MAO exists in at least two major forms (Johnston, 1968; Fuller, 1972), types A and B, which differ in their substrate specificity, sensitivity to inhibitors and location within the brain (Youdim and Findberg, 1983). Thus, MAO-A preferentially deaminates 5-HT, NA and AD, while MAO-B deaminates non-polar amines such as benzylamine, beta-phenylethylamine, phenylethanolamine and tryptamine. DA and tyramine are substrates for both subtypes of the enzyme (Fuller, 1972; Cooper et al., 1978; Youdim and Findberg, 1983). In addition, MAO-A activity is thought to be predominantly, but not exclusively, associated with the neuron and MAO-B activity with extraneuronal cells (Youdim and Findberg, 1983). It should be noted, however, that the characteristics of the enzymes are not the same in all tissues and species. Thus, in human brain cortex and caudate nucleus DA is predominantly deaminated by MAO-B, while in rat brain caudate DA is deaminated by MAO-A (Garrick et al., 1979; Youdim and Findberg, 1983). Furthermore, MAO-A appears to be the predominant form present in rodent brain, including rat, mouse and hamster, while MAO-B predominates in primate and human brain (Garrick et al., 1979).

1.4.2.2 Inhibitors of Monoamine Oxidase

The three main classes of the MAOI's are the hydrazines (e.g. iproniazid, phenelzine, isocarboxazide, nialamide), the cyclopropylamines (e.g. tranylcypramine) and the acetylenic propargylamines (e.g. clorgyline, deprenyl, pargyline) (Youdim and Findberg, 1983). All of these compounds inhibit MAO irreversibly.

The hydrazines and tranylcypramine are non-selective inhibitors of MAO, while clorgyline is selective for MAO-A, and deprenyl (also known as selegeline) and pargyline are selective for MAO-B (Johnston, 1968; Fuller, 1972; Youdim and Findberg, 1983).

In 1957 iproniazid was introduced into the clinic for the treatment of depression, and other non-selective MAOI's soon followed. The MAOI's exhibit a different therapeutic spectrum from other psychotropic drugs, and are demonstrably effective in neurotic depression and anxiety, mixed anxiety-depression and hypochondriasis, and phobic anxiety (particularly agrophobia), but not psychotic depression (Paykel, 1979a; Tyrer, 1979). Furthermore, MAOI's may often be effective when other treatments (e.g. with TCA's) have failed (Tyrer, 1979). However, conflicting data concerning their efficacy, concern over their safety, and the introduction of the TCA's (see section 1.4.3) led to a decline in their clinical use. The major clinical cause for concern was, and remains, the so-called "cheese reaction". This severe drug-induced reaction is a hypertensive crisis which occurs following ingestion of foods or medication containing high concentrations of tyramine or related sympathomimetic amines (Green and Costain, 1981; Youdim and Findberg, 1983). Tyramine, a substrate for both forms of MAO, is normally metabolized by MAO-A in the gut (Youdim and Findberg, 1983). However, following non-selective inhibition of MAO, tyramine is taken up by the vascular noradrenergic nerve endings where it displaces NA, thereby inducing the hypertensive reaction. In addition to the elevated blood pressure and, possibly, increased pulse rate, patients commonly experienced nausea and vomiting. Generally patients recover but occasionally angina, cardiac arrest,

pulmonary oedema, intracranial and subarachnoid haemorrhage occur (Kline and Cooper, 1980). Antidepressant treatment with MAOI's can be associated with numerous side effects and adverse reactions. The acute toxic effects observed following overdose include agitation, hallucinations, hyperpyrexia, hyperreflexia, changes in blood pressure, and convulsions. During long term therapy autonomic side effects include dry mouth, constipation, dizziness, postural hypotension, impotence and delayed ejaculation, while CNS effects include agitation, insomnia, irritability and motor restlessness (Kline and Cooper, 1980). In some cases MAOI's have been shown to convert a retarded depression into an agitated depression. In addition, hypomania or precipitation of psychosis in patients with a history of schizophrenia has been reported. The incidence of liver toxicity with iproniazid, manifest by jaundice, hepatocellular damage, elevated liver enzymes and biliary stasis, resulted in the withdrawal of iproniazid as an antidepressant (Kline and Cooper, 1980).

Renewed interest in the use of MAOI's for the treatment of depression followed the identification of the two subtypes of MAO and the introduction of the selective inhibitors clorgyline and deprenyl (Johnston, 1968; Fuller, 1972). If one accepts that 5-HT and/or NA deficiency play important roles in depression and since both 5-HT and NA are preferred substrates for MAO-A, then it may be argued that selective inhibitors of MAO-A would be preferred to selective inhibitors of MAO-B. However, since gut MAO is predominantly type A then administration of selective MAO-A inhibitors would still render the patient susceptible to the "cheese reaction". Both clorgyline and deprenyl exhibit antidepressant efficacy (see Fuller, 1981, and

Mann et al., 1984, and references cited therein), although whether MAO-A inhibitors exhibit increased efficacy over MAO-B inhibitors, or whether MAO-B inhibitors, which would not be expected to render the patient susceptible to the "cheese reaction", possess any increased safety advantages over selective inhibitors of MAO-A, remains a topic of debate (Mann et al., 1984).

A more recent approach has been the development of rapidly reversible selective MAO-A inhibitors such as moclobemide, MD 780515 and FLA 336(+). In animal studies these compounds, unlike clorgyline or the non-selective irreversible MAOI's, do not appear to potentiate tyramine sensitivity and, furthermore, moclobemide exhibits antidepressant efficacy with apparent lower potential for tyramine potentiation (Mann et al., 1984). Whether such compounds prove to possess any advantages, in terms of efficacy and safety, over clinically available antidepressants remains to be evaluated.

In animal studies acute administration of MAOI's results in the accumulation of 5-HT, NA and DA together with a reduction in the respective metabolite levels (Youdim and Findberg, 1983; Willner, 1985) consistent with reduced MAO activity. Furthermore, acute treatment with MAOI's demonstrably potentiates and increases the duration of effect of 5-HT-induced behaviours (Ortmann et al., 1980). While MAO inhibition may be achieved with low doses of these agents, higher doses may cause other pharmacological effects such as inhibition of monoamine uptake and receptor blockade, although these effects are thought to be secondary to inhibition of MAO activity in terms of their ability to elevate synaptic neurotransmitter levels and their antidepressant effect (Kline and Cooper, 1980; Youdim and

Findberg, 1983). Current evidence suggests that up to 80% of MAO must be inhibited before any behavioural or therapeutic effects are observed (Kline and Cooper, 1980; Green and Costain, 1981), and while this level of MAO inhibition and peak brain amine levels may be readily attained, i.e. within 7 days (Robinson et al., 1979), such effects would not appear to correlate with the latency for clinical response, where at least 2-4 weeks of treatment are required before any therapeutic benefit is observed (Oswald et al., 1972; Kline and Cooper, 1980; Green and Costain, 1981). Moreover, Robinson et al. (1979) demonstrated that during chronic MAOI treatment of rodents brain amine and metabolite levels returned to near pre-treatment levels at a time equivalent to the onset of clinical remission in patients. Furthermore, the same studies suggest that chronic treatment with some, but not all, MAOI's results in increased activity of the enzymes involved in monoamine synthesis (see also Campbell et al., 1979), while other workers suggest that amine synthesis (especially that of 5-HT) may be reduced following chronic MAOI treatment (see Youdim and Findberg, 1983, and Willner, 1985, and references cited therein). Such observations suggest that the antidepressant effect of MAOI's is not due directly to inhibition of MAO activity per se nor the resultant increase in neurotransmitter levels, but rather to secondary adaptive changes that are induced in response to the initial abnormally high levels of central 5-HT, NA and, possibly, DA. The adaptive changes in neurotransmitter function following chronic MAOI treatment will be discussed in section 1.4.5.

1.4.2.3 Inhibitors of Monoamine Oxidase and Monoamine Precursors

L-Tryptophan, but not L-DOPA, has been reported to potentiate the

antidepressant effect of MAOI's (see Green and Costain, 1981, van Praag, 1981b, and Mann et al., 1984). The antidepressant effect of the serotonin precursors L-tryptophan or 5-HTP, with or without concomitant MAOI treatment, together with the lack of antidepressant effect of L-DOPA (see Goodwin et al., 1970), has often been quoted in support of the causative role of a deficiency of 5-HT, rather than of the catecholamines, in depression (Coppen, 1967, Murphy et al., 1978; van Praag, 1979, 1981a, 1981b; van Praag et al., 1987), and consequently has spurred research into "5-HT specific" antidepressants. Since 5-HT and NA are both preferred substrates for MAO-A, attention has focused on the development of drugs which specifically inhibit the re-uptake of 5-HT, rather than the catecholamines, in an attempt to increase 5-HT function as opposed to attempting specifically to inhibit MAO activity associated with serotonin neurons (see section 1.4.3.2). Whether the rationale for the development of 5-HT-specific antidepressants is justified is open to question, however, since the combination of L-tryptophan with MAOI's also increases tryptamine levels in central catecholamine neurons, where it displaces the catecholamines thereby increasing central catecholamine turnover (Green and Costain, 1981). Furthermore, whether the increased 5-HT turnover observed following serotonin precursor treatment is indicative of increased serotonergic function is debatable since intraneuronal metabolism of 5-HT by MAO may account for the increased 5-HIAA levels without increased 5-HT release (Green and Grahame-Smith, 1975). In addition, while serotonin precursor treatment has been claimed to be antidepressant in patients that exhibit low CSF 5-HIAA levels (van Praag, 1979, 1981b), whether administration of L-tryptophan or 5-HTP alone are indeed antidepressant in all depressed patients is equivocal and a

topic of debate (see Murphy et al., 1978). The efficacy of serotonin precursors may therefore simply serve to identify a "5-HT-deficient subgroup" of depressives (van Praag, 1981b), and thus demonstrate the heterogeneity of the depressed patient population.

1.4.3 Monoamine Re-Uptake Inhibitors

During clinical investigation of a series of phenothiazine analogues for sedative or hypnotic properties, Kuhn (1958) observed that imipramine, a dibenzazepine compound differing from the phenothiazines only by replacement of a sulphur atom with an ethylene linkage, was relatively ineffective in quieting psychotic patients, but demonstrated beneficial effects when administered to certain depressed patients. This observation led to the development of other chemically related "tricyclic" antidepressants (TCA's). It is now generally accepted that one important mechanism of action of the TCA's is their ability to inhibit the re-uptake processes of 5-HT and/or NA (see Carlsson, 1970).

1.4.3.1 5-HT and NA Re-uptake Processes

Since the early 1970's it has become accepted that associated with neurons are specific transport systems for the particular transmitter synthesized and released by those neurons. Thus, neurons which release NA possess a specific transport system capable of transporting released NA back into the cytoplasm of the neuron, whereupon it is either metabolized intraneuronally, or stored in granules where it becomes available for further release. Similar specific uptake systems are associated with dopaminergic and serotonergic neurons (Iversen, 1975). The re-uptake processes are

the primary method of removal (and hence inactivation) of the neurotransmitter from the synapse (Iversen, 1975; Cooper et al., 1978; Green and Costain, 1981). The evidence for the re-uptake processes of the monoamine neurotransmitters have been extensively reviewed by Iversen (1975), and will only be briefly described here.

Autoradiographic and histochemical studies have indicated that the uptake and retention of exogenous NA occurs primarily in postganglionic sympathetic neurons or in catecholamine-containing neurons in the CNS. In addition, the ability to accumulate exogenous catecholamine appears to be a property of all parts of catecholaminergic neurons, including the preterminal area and cell body. The uptake of catecholamines appears to be an active process since it proceeds against a concentration gradient. Thus, in vitro studies have demonstrated that various tissues, including brain, are able to concentrate NA from the incubation medium. The properties of the NA uptake system in central NA neurons appear to be similar, if not indistinguishable, from those of the peripheral sympathetic neurons (Iversen, 1975). The uptake process for NA is saturable, and temperature and sodium dependant. Thus, like other similar processes, the NA uptake process may be inhibited by agents such as ouabain which block the NA^+/K^+-ATPase (sodium pump). The affinity of NA for the uptake system is high, although a variety of amines structurally related to NA may serve as alternative substrates, but with lower affinity, for the NA uptake system. Furthermore, in most species, the uptake process exhibits stereochemical selectivity in favour of the naturally occurring (-)-isomer of NA.

Central serotonergic neurons possess their own uptake system for 5-HT (Iversen, 1975). Thus, biochemical or autoradiographic studies indicate 5-HT accumulation in serotonergic neurons following administration of 5-HT into the CSF. Like the NA uptake system, the process of 5-HT uptake exhibits high affinity for 5-HT, is saturable and both temperature and sodium dependant, and may be inhibited by ouabain. Unlike the NA uptake system, however, the 5-HT uptake system is immediately inhibited by metabolic inhibitors (Iversen, 1975). The study of 5-HT uptake is complicated by the fact that the amine is readily taken up into catecholaminergic neurons by the NA and DA uptake systems. Thus, the uptake of 5-HT into brain slices or synaptosomes exhibits two saturable components; a high affinity uptake into serotonergic neurons and a lower affinity uptake into catecholaminergic neurons. However, with the exception of sympathetic nerve terminals in the pineal gland, it is unlikely that central catecholaminergic neurons are exposed to high concentrations of 5-HT, so, under normal conditions, the physiological relevance of 5-HT uptake into central catecholaminergic neurons is questionable.

The uptake process of exogenous monoamines by storage vesicles appears to be different from those mediating uptake into nerve terminals. For the catecholamines at least the vesicle uptake system has a lower affinity (about 1000 times) and is not dependent on the presence of Na^+ or K^+ ions (Iversen, 1975). Furthermore, the vesicle uptake systems are insensitive to many of the inhibitors which block the neuronal uptake processes.

Exogenous monoamines may also be taken up into non-neuronal tissues. The properties of these uptake processes are different from those of the neuron terminal. Thus, extraneuronal uptake of NA demonstrates no stereochemical selectivity for the (-)- and (+)-isomers of NA, and, while it is a saturable process, it displays much lower affinity for the catecholamine substrates (Iversen, 1975). Furthermore, potent inhibitors of neuronal catecholamine re-uptake are relatively less potent on extraneuronal catecholamine uptake.

1.4.3.2 Inhibitors of 5-HT and NA Re-uptake Processes

It is accepted that the high affinity energy dependant re-uptake systems for 5-HT, NA and DA are the most important mechanisms by which the synaptic action of these neurotransmitters is terminated (Iversen, 1975; Fuller and Wong, 1977). Drugs which block the re-uptake processes will therefore prolong the actions of these monoamines in the CNS. The relative potencies of the TCA's to inhibit 5-HT, NA and DA re-uptake have been thoroughly investigated (see Waldmeier et al., 1976; Iversen and Mackay, 1979, and references cited therein; Sulser and Mobley, 1980) and these compounds exhibit a spectrum of activity across all three monoaminergic systems. The TCA's include imipramine, desmethylimipramine (desipramine), amitriptyline, nortriptyline, 3-chloroimipramine (clomipramine), doxepin and diothepin. The structure-activity relationship of the TCA's has been reviewed by Zeelen (1980). Most conventional TCA's potently inhibit 5-HT and NA re-uptake by central serotonergic and noradrenergic neurons respectively, but exhibit relatively lower potency to inhibit DA re-uptake by central dopaminergic neurons (see Sulser and Mobley, 1980). Thus, the ability of the TCA's to inhibit 5-HT and NA

re-uptake, as opposed to DA re-uptake, by monoaminergic neurons in the CNS is the most outstanding pharmacological activity shared by these compounds, and is thought to be integral to their efficacy as antidepressants (Coppen, 1967; Schildkraut and Kety, 1967; Carlsson, 1970; Sulser et al., 1978; Sulser and Mobley, 1980; Sugrue, 1981a, 1981b). Furthermore, monoamine re-uptake inhibition is the key property by which new antidepressants have been identified, and a number of compounds have been developed which reportedly have highly selective actions on 5-HT or NA re-uptake.

While the MAOI's are more effective in neurotic depression, the TCA's appear to be more effective in patients with psychotic or endogenous patterns of depression provided the illness is neither severe nor associated with delusions (Paykel, 1979a). All of the TCA's have similar unwanted side effects, the most frequent of which are dryness of mouth, difficulty with visual accommodation and in initiating micturition, constipation, palpitations and postural hypotension (Mindham, 1979), while urinary retention, paralytic ileus and heart failure are occasionally observed. Other side effects include sweating, dizziness, tremor, ataxia, Parkinsonism, lactation, oedema and skin rashes, while drowsiness may also be experienced during the first few days of treatment (Mindham, 1979). The severity of the side effects vary according to the drug preparation employed. The majority of the side effects are indicative of anticholinergic properties; indeed the TCA's are potent ligands for the muscarinic cholinergic receptor as determined by [^{3}H]-quinuclidinylbenzilate ([^{3}H]-QNB) binding (Peroutka and Snyder, 1980; Green and Nutt, 1983). Furthermore, the majority of TCA's show similar potency ratios for the histamine H_1 receptor

(determined by [^{3}H]-mepyramine binding) as for the muscarinic receptor (Hall and Ogren, 1981; Green and Nutt, 1983), and potently inhibit histamine-sensitive adenylate cyclase (see Green and Costain, 1981). It is possibly the anti-histaminic property that is related to the sedative side effects of these compounds (Green and Nutt, 1983). It is unlikely, however, that the anticholinergic or antihistaminic properties of these drugs are related to their therapeutic efficacy since neither the classical anticholinergics nor potent histamine antagonists have been reported to possess antidepressant properties (Green and Nutt, 1983).

During the last decade several compounds have been developed which display greater selectivity between the 5-HT and NA re-uptake processes. These compounds are generally dissimilar in structure from the TCA's. Those compounds which selectively inhibit 5-HT re-uptake (as determined by inhibition of [^{3}H]-5-HT uptake in synaptosomes or blockade of the p-chloroamphetamine-induced depletion of brain 5-HT) include citalopram, femoxetine, fluoxetine, fluvoxamine, paroxetine and zimelidine (Iversen and Mackay, 1979; Green and Costain, 1981; Shopsin et al., 1981; Hyttel, 1982; Harms, 1983; Norman and Burrows, 1983; Thomas et al., 1987), while those selective for the NA re-uptake process (as determined by the inhibition of [^{3}H]-NA uptake in synaptosomes or blockade of the 6-OHDA-induced depletion of NA) include maprotiline, nisoxetine, nomifensine and viloxazine (Gerhards et al., 1974; Iversen and Mackay, 1979; Green and Costain, 1981; Shopsin et al., 1981; Norman and Burrows, 1983).

Acute treatment with the TCA's or the more recent monoamine-specific re-uptake inhibitors reverses the behavioural syndrome induced following depletion of central monoamine stores with reserpine or tetrabenazine (Moller Nielsen, 1980), and this effect is thought to be indicative of the re-uptake inhibiting properties of these compounds. However, it cannot be assumed that blockade of monoamine re-uptake results in increased stimulation of post-synaptic monoamine receptors. It is generally accepted that acute treatment with either the TCA's or the 5-HT- or NA-specific compounds reduces both the firing rate of the respective central monoamine neurons and the rate of monoamine synthesis (Bymaster and Wong, 1974; Gallager and Aghajanian, 1974; Fuller and Wong, 1977; Carlsson and Lindqvist, 1978; Green and Costain, 1981; Fuller, 1985; Willner, 1985). This effect is almost certainly due to a regulatory feedback mechanism resulting from an enhanced action of the monoamine at the respective pre-synaptic receptors (see Fuller and Wong, 1977; Green and Costain, 1981). Furthermore, inhibition of monoamine re-uptake would be expected to potentiate 5-HT- or NA-mediated behaviours. However, the majority of monoamine re-uptake inhibitors also interact strongly with post-synaptic receptors (see Peroutka and Snyder, 1980). It is apparent that several antidepressant drugs have affinities for 5-HT_2 binding sites labelled by [^{3}H]-spiperone (see Peroutka and Snyder, 1979) <u>in vitro</u> at concentrations in the same range as those affecting amine re-uptake (Ogren and Fuxe, 1985). Thus the ability of some monoamine re-uptake inhibitors (notably amitriptyline, desipramine, imipramine and nortriptyline) to block certain 5-HT-mediated behaviours, e.g. L-5-HTP- and d-LSD-induced head twitch in mice and the potency of 5-HT antagonists to block tryptamine-induced clonic seizures in rats, have been shown

to correlate with their potency to displace [^{3}H]-spiperone *in vitro*, indicative of 5-HT_2-receptor blocking activity in the CNS (see Leysen, 1985; Ogren and Fuxe, 1985). Several monoamine re-uptake inhibitors may therefore also be considered as 5-HT_2 antagonists (for the classification of 5-HT receptors the reader is directed to Bradley et al., 1986). Exceptions to this are the specific 5-HT re-uptake inhibitors which have low affinity for 5-HT_1 and 5-HT_2 binding sites labelled by [^{3}H]-d-LSD, 5-HT_2 binding sites labelled by [^{3}H]-spiperone or 5-HT_1 binding sites labelled by [^{3}H]-5-HT (Peroutka and Snyder, 1979), e.g fluoxetine and zimelidine (see Wong et al., 1983; Ogren and Fuxe, 1985; Willner, 1985). While such compounds demonstrably potentiate L-5-HTP-induced behaviours (Ortmann et al., 1980) this ability is only demonstrated by those monoamine re-uptake inhibitors which also possess marked affinity for post-synaptic 5-HT_2 receptors at doses far higher than would be expected from their potency on 5-HT re-uptake (cf. Moller Nielsen, 1980, and Wong et al., 1983). In addition, a number of monoamine re-uptake inhibitors also show marked affinity for $alpha_1$- and $alpha_2$-adrenoceptors (Hall and Ogren, 1981; Wong et al., 1983) and consequently may modify clonidine-induced hypoactivity (Heal et al., 1983). In acute studies therefore the action of these compounds on monoamine re-uptake mechanisms and the blockade of post-synaptic receptors results in functionally opposite effects, and which effect predominates ultimately determines the effect of such compounds on serotonin-mediated behaviours.

As experienced with the MAOI's, clinical response to the monoamine re-uptake inhibitors is not observed until at least 2-4 weeks of drug treatment (Oswald et al., 1972). Thus inhibition of 5-HT and/or NA

re-uptake following acute treatment alone would not account for the antidepressant efficacy of these compounds. Furthermore, not all compounds which inhibit monoamine re-uptake are antidepressant. For example, cocaine, a moderately potent inhibitor of NA re-uptake (see Sulser and Mobley, 1980) does not possess any antidepressant activity (Green and Costain, 1981). It therefore seems more likely that the antidepressant effect of the monoamine re-uptake inhibitors is due to secondary adaptive changes induced in response to the initial elevated levels of 5-HT and NA.

1.4.4 Atypical Antidepressants

The atypical antidepressants are those compounds which are demonstrably antidepressant in the clinic but possess little or no inhibitory action on MAO activity or monoamine re-uptake. The two principal members of this group of compounds are iprindole and mianserin, although flupenthixol, a D_1-DA antagonist (Seeman, 1981) and the $beta_2$-adrenoceptor agonist salbutamol have both been reported to possess antidepressant properties (see Iversen and Mackay, 1979; Mindham, 1979), and thus may be categorised as members of the atypical antidepressants.

Iprindole, in comparison to the monoamine re-uptake inhibitors, has little effect on serotonin or catecholamine re-uptake *in vitro* or *in vivo* (Rosloff and Davis, 1974; Sulser and Mobley, 1980; Hyttel, 1982), and is only weakly effective in reversing the behavioural effects of reserpine or tetrabenazine treatment in rodents (Moller Nielsen, 1980). Furthermore, iprindole exhibits little affinity for central monoamine receptors, indeed the binding data of Peroutka and Snyder (1980) and Hall and Ogren (1981) suggest that iprindole

has a higher affinity for H_1 histamine receptors than for other various monoamine receptors. In comparison to the MAOI's or the monoamine re-uptake inhibitors, the lack of observable acute effect of iprindole on central neurotransmitter systems makes it an unlikely candidate for an antidepressant; however, iprindole exhibits clinical antidepressant efficacy (Hicks, 1965; Daneman, 1967; Sterlin et al., 1968).

The antidepressant potential of mianserin was initially predicted on the basis of the similarity of its effect on the human encephalogram to that seen with the TCA's (see reviews by Itil and Soldatos, 1980, and Itil, 1983), and its antidepressant efficacy has been confirmed in a number of studies (see review by Brogden et al., 1978, and section 3.3.4). The most frequently reported side effect with mianserin is drowsiness which, as with the TCA's, is usually transient and disappears after the first few days of treatment (see Jaskari et al., 1977). Mianserin is at best only moderately active as an inhibitor of 5-HT or NA re-uptake in vitro (Hyttel, 1982) and essentially devoid of effect on 5-HT re-uptake in vivo (Goodlet et al., 1977). Its ability to reduce brain concentrations of NA and increase central NA turnover is thought to be due to antagonist activity at central pre-synaptic $alpha_2$-adrenoceptors (Baumann and Maitre, 1977). It should be noted that the catecholamines, like 5-HT (Moret, 1985), are able to control their own release via an action on autoreceptors located on the pre-synaptic nerve terminals and cell bodies of catecholaminergic neurons (see Starke, 1981; Chesselet et al., 1984; and the electrophysiological studies of Aghajanian and co-workers reviewed by Aghajanian and Rogawski, 1983). In addition, mianserin also possesses marked affinity for

central H_1 histamine receptors, alpha$_1$-adrenoceptors and 5-HT_2 receptors (see Wong et al., 1983), and also exhibits antagonist activity at 5-HT_{1c} (as defined by Pazos et al., 1985) receptors (Fozard, 1987) and at 5-HT receptors in the periphery (Saxena et al., 1971). As with the TCA's, the anti-histaminic properties of mianserin may account for the incidence of drowsiness observed in the clinic. Furthermore, in comparison to the TCA's, the reduced frequency of reported anti-cholinergic side effects corresponds with its lower affinity for central muscarinic receptors (cf. Jaskari et al., 1977, and Wong et al., 1983).

The atypical antidepressants, like other compounds used in the treatment of depression, only exhibit antidepressant efficacy after 2-4 weeks of treatment, and thus any direct effect of these compounds following acute treatment may not be directly attributable to their antidepressant efficacy.

1.4.5 Biochemical changes following chronic antidepressant treatment

As indicated throughout the preceding sections there is generally no correlation between the acute effects of all the currently available antidepressant drugs on central neurotransmitter systems to the latency of their antidepressant effect observed in the clinic. Such observations demonstrate that chronic treatment with these compounds is a prerequisite for clinical efficacy, indicating that long-term modification of central neurotransmitter function induced by chronic antidepressant treatment is necessary for the remission from depression.

In rats, chronic treatment with the MAOI's phenelzine and tranylcypromine leads to an initial increase in central 5-HT, NA and DA levels, but is followed by a gradual decline in the brain monoamines towards control levels with continued treatment (Robinson et al., 1979). Furthermore, the same studies showed that chronic phenelzine treatment was associated with an adaptive increase in tryptophan hydroxylase activity, while chronic tranylcypromine treatment was associated with an increase in aromatic amino acid decarboxylase activity. No change in tyrosine hydroxylase activity was observed during chronic treatment with either drug. In another series of experiments by this group (see Campbell et al., 1979) chronic treatment with clorgyline resulted in a persistent elevation of brain NA levels while the initial rise in 5-HT levels returned to control levels by 21 days of treatment. Conversely, chronic treatment with high, but not low, doses of pargyline led to increased 5-HT levels following 21 days of treatment only, but had little effect on NA levels throughout the duration of treatment. Neither clorgyline or pargyline treatment had any significant effect on tyrosine hydoxylase or tryptophan hydroxylase activity or on 5-HIAA levels. The electrophysiological studies of Blier et al. (1986) indicated that continuous exposure to clorgyline, but not pargyline or deprenyl, reduced the firing rate of 5-HT, but not NA, neurons. Furthermore, the stimulation-induced suppression of 5-HT neuron firing rate was increased by clorgyline and pargyline, but not by deprenyl, while none of the treatments modified the stimulation-induced suppression of NA neuron firing rate (Blier et al., 1986). While acute treatment with the TCA's generally reduces brain NA turnover, long-term administration tends to increase NA turnover (as indicated by increased MHPG levels), probably via pre-

and post-synaptic feedback mechanisms (Roffman et al., 1977; Sugrue, 1980, 1981a, 1981b). In contrast however, both acute and chronic treatment with the TCA's or 5-HT selective re-uptake inhibitors generally have the same effect on 5-HT turnover. Thus desipramine does not affect 5-HT turnover acutely nor following chronic treatment, while clomipramine and zimelidine both decrease 5-HT turnover when given either acutely or chronically (Sugrue, 1981b; Willner, 1985). It is thought that any reduction in neurotransmitter synthesis or neuronal activity following chronic antidepressant treatment are indicative of partial compensation in response to excessive monoamine levels induced by MAO inhibition or inhibition of NA and/or 5-HT re-uptake (Willner, 1985), while the differences between the effects of chronic treatment with particular drugs are more likely to reflect differences in the feedback mechanisms of the neurotransmitter systems.

The atypical antidepressants mianserin and iprindole have different effects on NA and 5-HT when given either acutely or chronically. Mianserin appears to increase NA, but not 5-HT, turnover following acute and chronic administration, while, conversely, iprindole only increases 5-HT turnover following chronic treatment (Sugrue, 1980, 1981b; Willner, 1985).

Current data therefore indicate a general lack of common effect on the turnover of central 5-HT and NA in rodents following chronic treatment with the various antidepressant drugs. This lack of common effect may, in part, also be explained by the ability of these drugs to interact with central pre- and post-synaptic 5-HT and NA receptors, and with other neurotransmitter systems, which may

modify either the induction or expression of various compensatory processes in response to changes in the synaptic concentrations of 5-HT and/or NA.

Various antidepressants (especially the TCA'S and mianserin) exhibit marked affinity for central neurotransmitter receptors (see sections 1.4.3.2 and 1.4.4). Consequently investigators have studied the effect of chronic antidepressant treatment on the binding characteristics of central neurotransmitter receptors.

While both the TCA's and atypical antidepressants generally exhibit low affinity for displacing [^{3}H]-dihydroalprenalol ([^{3}H]-DHA) from central beta-adrenoceptors (Hall and Ogren, 1981), chronic, but not acute, treatment with most TCA's leads to a reduction in the number of beta-adrenoceptors without modifying the affinity of the ligand (Banerjee et al., 1977; Peroutka and Snyder, 1980; Sellinger-Barnette et al., 1980; Green and Nutt, 1983). Furthermore, the ability to down-regulate beta-adrenoceptors, following chronic treatment only, appears to be a feature also shared by the MAOI's (including clorgyline, but not deprenyl, suggesting that continued inhibition of MAO-A may be an important feature of this effect), iprindole and electroshock, but possibly not mianserin (Banerjee et al., 1977; Sellinger-Barnette et al., 1980; Sethy and Harris, 1981; Finberg and Youdim, 1983; Green and Nutt, 1983; Kellar and Bergstrom, 1983; Sulser, 1983; Sulser et al., 1983). Interestingly chronic treatment with cocaine, which inhibits NA re-uptake but exhibits no antidepressant properties, or other non-antidepressant psycho-active compounds including examples of antipsychotics, anxiolytics, barbituates,

anticholinergics and psycho-stimulants, do not down-regulate beta-adrenoceptors (Sellinger-Barnette et al., 1980; Sethy and Harris, 1981). Whether the ability to down-regulate beta-adrenoceptors per se is important for the antidepressant effect of these compounds is questionable since the 5-HT selective re-uptake inhibitors, fluoxetine and zimelidine, generally do not induce this response following chronic treatment (Peroutka and Snyder, 1980; Green and Nutt, 1983). In one study, however, Sethy and Harris (1981) observed that continuous infusion with zimelidine for 7 days reduced beta-adrenoceptor number in rat cortex, indicating that under the right conditions 5-HT-selective re-uptake inhibitors may also possess the property of inducing beta-adrenoceptor down-regulation. Chronic treatment with the $beta_2$-adrenoceptor agonist, clenbuterol, has also been observed to down-regulate central beta-adrenoceptors (Frazer et al., 1986). Current evidence indicates that beta-adrenoceptors are coupled to a stimulatory guanine nucleotide-binding protein through which adenylate cyclase activity is stimulated (Lefkowitz and Hoffman, 1980; Strosberg et al., 1982). Thus stimulation of beta-receptors in the CNS, either by the natural transmitter (NA) or beta-adrenoceptor agonists, leads to increased production of cyclic adenosine 5'-monophosphate (cAMP) (Sulser, 1978; Frazer et al., 1986). It is not surprising therefore that antidepressant treatments, including electroshock, which down-regulate beta-adrenoceptors also induce a subsensitivity of the NA-dependent adenylate cyclase system to stimulation by NA or isoprenaline (Vetulani et al., 1976a; Frazer and Mendels, 1977; Gillespie et al., 1979; Green and Nutt, 1983; Sulser, 1983). Interestingly, chronic treatment with mianserin, nisoxetine (a NA-selective re-uptake inhibitor) or zimelidine all reduce the

sensitivity of the NA-dependent adenylate cyclase system under treatment conditions which did not reduce the density of central beta-adrenoceptors (Mishra et al., 1980; Green and Nutt, 1983; Sulser, 1983). Conversely, chronic treatment with fluoxetine neither down-regulates beta-adrenoceptor binding nor reduces the sensitivity of the NA-dependent adenylate cyclase system (Peroutka and Snyder, 1980; Mishra et al., 1981). The antidepressant-induced subsensitivity of the NA-dependent adenylate cyclase system appears to be dependent on the maintained integrity of the noradrenergic input. Thus, Janowsky et al. (1982) showed that unilateral electrolytic lesions of the locus coeruleus, which reduced the NA (but not 5-HT) content of the ipsilateral frontal cortex, abolished the desipramine-induced reduction in the cAMP response to NA in slices of the ipsilateral frontal cortex. Conversely, chronic desipramine treatment still reduced the cAMP response to NA in slices of the contralateral (non-lesioned) frontal cortex. These observations suggest that the reduced sensitivity of the NA-dependent adenylate cyclase system may be induced in response to increased availability of NA rather than 5-HT. In support of this view, Mishra et al. (1981) demonstrated that raphe lesions which selectively reduced the level of 5-HT did not induce any change in the sensitivity of the NA-dependent adenylate cyclase system or the density of beta-adrenoceptor binding sites. In contrast, lesions of the medial forebrain bundle, which reduced the levels of 5-HT, NA and DA, also increased the responsiveness of the NA-dependent cyclase system but had no effect on the density of beta-adrenoceptors (Mishra et al., 1981). Conversely, Vetulani et al. (1976b) had previously shown that prolonged reduction in central monoamine availability induced by chronic reserpine treatment, or chemical

sympathectomy with 6-hydroxydopamine (6-OHDA) increased the responsiveness of the NA-dependent adenylate cyclase system. The same studies showed that protection of noradrenergic neurons against the neurotoxic action of 6-OHDA afforded by desipramine treatment also prevented the development of increased responsiveness of the NA-dependent adenylate cyclase system. Thus the 6-OHDA-induced changes in the reactivity of the cAMP generating system appear to be related to changes in the availability of NA rather than that of other neurotransmitters. Prolonged elevation or reduction of central NA availablility therefore leads to a compensatory decrease or increase in the responsiveness of the NA-dependent adenylate cyclase system, respectively. It may be argued that the ability of some antidepressants that exhibit a margin of 5-HT selectivity, but which also reduce the reactivity of the NA-dependent cAMP generating system, may preclude such a conclusion. For example, the TCA's with terminal tertiary amines show marginal selectivity in their ability to inhibit 5-HT, rather than NA, re-uptake in vitro (Iversen and Mackay, 1979). The in vivo conversion of compounds such as amitriptyline and clomipramine to their secondary amine metabolites (nortriptyline and desmethylclomipramine, respectively), however, results in increased selectivity for NA, rather than 5-HT, re-uptake (Fuller and Wong, 1977). The metabolites of these compounds may therefore be ultimately responsible or the apparent ability of amitriptyline and clomipramine to induce the subsensitivity of the NA-dependent adenylate cyclase system in vivo (Mishra et al., 1981).

Generally the studies cited above indicate that at least 2-4 weeks of continuous antidepressant treatment is required to induce the

decrease in beta-adrenoceptor number or reduce the responsiveness of the NA-dependent adenylate cyclase system. The studies of Wiech and Ursillo (1980) suggest that the rate of desipramine-induced decrease in central beta-adrenoceptor number may be accelerated by concomitant blockade of the alpha$_2$-mediated negative feedback system with the alpha$_2$-antagonist yohimbine. Thus it may be argued that the antidepressant-induced down-regulation of beta-adrenoceptors and/or sensitivity of the NA-dependent adenylate cyclase system only follows a desensitization of the alpha$_2$-mediated negative feedback system, which would provide a possible explanation of the latency of antidepressant efficacy experienced in the clinic. In support of this view, Smith et al. (1981) demonstrated that chronic treatment with amitriptyline decreased [^{3}H]-clonidine binding to pre-synaptic (sic) central alpha$_2$-adrenoceptors. Furthermore, Heal et al. (1987) have shown that repeated electroshock or chronic desipramine treatment reduced the number of [^{3}H]-idaxozan binding sites, which are believed to be predominantly post-synaptic alpha$_2$-adrenoceptor binding sites. In contrast however, Sethy et al. (1983) demonstrated that continuous infusion with a broad spectrum of antidepressant drugs, including amitriptyline, desipramine, iprindole, mianserin, nomifensine and zimelidine, for 7 days, failed to decrease the number of [^{3}H]-clonidine binding sites. The effect of chronic antidepressant treatment on the number of central alpha$_2$-binding sites is therefore equivocal. However, the behavioural data of Heal et al. (1983, 1987) indicates that chronic amitriptyline, desipramine or mianserin treatment, or indeed repeated electroshock, induces a progressive decrease in clonidine-induced hypoactivity in rats; indicative of an adaptive decrease in the function of central alpha$_2$-adrenoceptors. In

accordance with these animal studies, Smith et al. (1983) observed a decrease in the number of [^{3}H]-clonidine binding sites on blood platelet membranes following treatment of endogenously depressed patients with TCA's or ECT.

A number of antidepressants, e.g. amitriptyline, clomipramine, desipramine, imipramine, mianserin and nortriptyline, exhibit relatively high affinities for central alpha$_1$-adrenoceptors labelled with [^{3}H]-WB4101 (Peroutka and Snyder, 1980; Hall and Ogren, 1981). Chronic antidepressant treatment, however, generally has no effect on either the number of alpha$_1$-adrenoceptors nor the affinity of the antidepressants for these binding sites labelled with either [^{3}H]-WB4101 (Peroutka and Snyder, 1980) or [^{3}H]-prazosin (Menkes et al., 1983; Mogilnicka et al., 1987). Chronic antidepressant treatment, however, does appear to enhance the ability of the alpha$_1$-agonist, phenylephrine, to compete for the sites labelled by [^{3}H]-prazosin (Menkes et al., 1983). Behavioural studies have also demonstrated that chronic antidepressant treatment potentiates both the exploratory activity of rodents induced by phenylephrine (principally following desipramine treatment; Mogilnicka et al., 1987) and aggressive behaviour in both mice (Maj et al., 1980) and rats (Maj et al., 1979) induced by acute treatment with clonidine and apomorphine, respectively. These results are interpreted as being indicative of an antidepressant-induced functional supersensitivity to alpha$_1$-adrenoceptor agonists. However, it should be noted that potentiation of phenylephrine-induced exploratory activity was not observed following chronic amitriptyline, imipramine or desipramine treatment, or following repeated electroshock, by the earlier study of Green et al. (1986).

The foregoing observations suggest that, generally, a common biochemical effect of chronic antidepressant treatment may be the down-regulation of central beta-adrenoceptors (and hence reduced sensitivity of the NA-dependent adenylate cyclase system), together with a desensitization of the $alpha_2$ receptor-mediated negative feedback system and, possibly, the increased agonist affinity of central $alpha_1$-adrenoceptors. Such observations may, at least in part, explain the antidepressant effect of these compounds, and furthermore, implies that the primary target of antidepressants may be the central noradrenergic system at the exclusion of other neurotransmitter (e.g. 5-HT) systems. It should be noted, however, that central noradrenergic and serotonergic systems are not independent. Indeed, Svensson et al. (1974) demonstrated that the clonidine-induced reduction the firing rate of NA neurons in the nucleus locus coeruleus was reflected in a concomitant reduction in the firing rate of 5-HT neurons in the nucleus raphe dorsalis. Furthermore, the response of the 5-HT neurons was dependent on the maintained integrity of the noradrenergic input to the raphe. Thus any overall change in the level of noradrenergic activity would be expected to be reflected by concomitant changes in the level of activity exhibited by other neurotransmitter systems on which noradrenergic systems impinge. In addition, Nimgaonkar et al. (1985) have shown that the down-regulation of rat cortical beta-adrenoceptors following chronic desipramine or clenbuterol treatment, or repeated electroshock, was abolished if serotonergic neurons were previously lesioned with the neurotoxin 5,7-dihydroxytryptamine. Conversely, desipramine and electroshock both down-regulated cortical beta-adrenoceptors following 5-HT synthesis inhibition with p-chlorophenylalanine (pCPA). These

observations indicate that the antidepressant-induced decrease in beta-adrenoceptor number is not dependent on an action of 5-HT per se, but, as suggested by Nimgaonkar et al. (1985), may be modulated by an unknown factor, normally present at 5-HT terminals in the cortex, which is removed by the neurotoxic lesion. The studies of Svensson et al. (1974) and Nimgaonkar et al. (1985) clearly demonstrate the interdependence of noradrenergic and serotonergic neurotransmitter systems in certain areas of the rodent CNS (see also Racagni et al., 1983).

Chronic treatment with examples of MAOI's (tranylcypromine, pargyline), TCA's (amitriptyline, imipramine, desipramine, clomipramine), the 5-HT selective re-uptake inhibitor, zimelidine, the atypical antidepressants (iprindole, mianserin), and the serotonin agonist trifluoromethylphenyl piperazine (TFMPP) generally reduce the number of 5-HT_2 receptors labelled with [^{3}H]-spiperone or [^{3}H]-ketanserin (TFMPP only) without affecting affinity (Peroutka and Snyder, 1980; Blackshear and Sanders-Bush, 1982; Anderson, 1983; Stolz et al., 1983; Blackshear et al., 1986; Green et al., 1986); mianserin also down-regulates 5-HT_2 receptor number following acute treatment (Blackshear and Sanders-Bush, 1982). Chronic treatment with fluoxetine has yielded equivocal results (cf. Peroutka and Snyder, 1980, and Stolz et al., 1983), while repeated electroshock invariably increases 5-HT_2 receptor number (Kellar and Bergstrom, 1983; Green et al., 1986). Green et al. (1986) and Stolz et al. (1983) have also demonstrated that the drug- or electroshock-induced change in 5-HT_2 receptor number is reflected by concomitant changes in the 5-HT_2 receptor-mediated head-twitch response in mice (see also Blackshear and Sanders-Bush, 1982) or the

behavioural syndrome in rats, respectively, induced by precursor loading or 5-HT agonist treatment. 5-HT_2 receptors are thought to mediate the ability of 5-HT to stimulate phosphotidylinsitol hydrolysis (Conn and Sanders-Bush, 1984). The antidepressant-induced reduction in 5-HT_2 receptor number, however, does not appear to be dependent on intact 5-HT neuron terminals. Clements-Jewery and Robson (1982) showed that pre-treatment of rats with the serotonergic neurotoxin, p-chloroamphetamine (pCA), did not inhibit the down-regulation of 5-HT_2 receptors. These authors suggest that their observations may indicate a possible post-synaptic locus of action for amitriptyline. However, an alternative explanation may be that other neurotransmitter systems are involved in the maintenance of 5-HT_2 receptor number.

Only chronic treatment with imipramine, MAOI's (especially those which inhibit MAO-A) or 5-HT agonists (e.g quipazine and TFMPP) reduce the number of 5-HT_1 receptors labelled with [^{3}H]-5-HT without affecting the affinity of the binding site for the ligand (Savage et al., 1979, 1980a, 1980b; Peroutka and Snyder, 1980), while other compounds (e.g. serotonin re-uptake inhibitors) have no effect (Peroutka and Snyder, 1980; Savage et al., 1980b; Stolz et al., 1983). Furthermore, drugs which deplete brain 5-HT (i.e. para-chlorophenylalanine (pCPA) or 5,7-dihydroxytryptamine (5,7-DHT), which alone increased the number of [^{3}H]-5-HT binding sites) prevented the clorgyline-induced reduction in [^{3}H]-5-HT number, indicating that the effect of the MAOI's is probably mediated by increased exposure of the 5-HT_1 receptors to 5-HT (Savage et al., 1980a). A number of 5-HT_1 receptor subtypes have been proposed (see review by Hartig, 1989). It has been suggested that 5-HT_{1A} or

5-HT_{1B} receptors (as defined by Pedigo et al., 1981), located on serotonergic cell bodies (Verge et al., 1986) or terminals (Engel et al., 1986) respectively, serve as 5-HT autoreceptors mediating the inhibitory effects of 5-HT on its own release (Moret, 1985). The 5-HT_{1A} site is thought to be linked to adenylate cyclase (Conn and Sanders-Bush, 1987). Pharmacological studies using the putative 5-HT_{1A} agonist, 8-OHDPAT (Tricklebank, 1985), however, have suggested that a functional change in pre-synaptic 5-HT_{1A} receptors follow chronic antidepressant treatment. Administration of 8-OHDPAT induces a hypothermic response in mice which is nearly abolished following chronic, but not acute, treatment with amitriptyline, desipramine, mianserin, zimelidine and tranylcypromine, and also after repeated electroshock (Green et al., 1986). Furthermore, chronic non-selective and chronic MAO-A inhibition reduces the ability of 8-OHDPAT to inhibit forskolin-stimulated adenylate cyclase activity indicative of down-regulation of 5-HT_{1A} receptors (Sleight et al., 1988). These observations suggest a possible down-regulation of pre-synaptic 5-HT_{1A} receptors following chronic antidepressant treatment. The recent discovery of apparent subgroups of central 5-HT receptors, and the development of specific ligands for these receptors, should provide the pharmacological tools by which the effect of chronic antidepressant treatment on the population and function of 5-HT receptor subtypes may be clarified.

Some workers have suggested that chronic antidepressant treatment may induce parallel changes in the sensitivity of gamma-aminobutyric acid (GABA) receptors to those observed for central beta-adrenoceptors. Chronic treatment with imipramine or nomifensine, or with the GABA agonists baclofen, progabide (which demonstrably increases NA

turnover; Lloyd et al., 1983) or 4,5,6,7-tetrahydroisoxazolo (5,4-C) pyridine-3-ol (THIP), all induce a reduction in beta-adrenoceptor number, labelled with [^{3}H]-DHA, in both rat cortex and hippocampus, while imipramine, nomifensine and THIP, but not baclofen, also induce a concomitant reduction in the number of high and low GABA receptors, labelled with [^{3}H]-GABA (Lloyd et al., 1983; Suzdak and Gianutsos 1985). Hill and Bowery (1981) have demonstrated that [^{3}H]-GABA labels a specific subgroup of GABA receptors, denoted $GABA_A$ receptors, while [^{3}H]-baclofen labels another subgroup of GABA receptors, denoted $GABA_B$ receptors. Furthermore, THIP has been shown to bind specifically to $GABA_A$ receptors (Hill and Bowery, 1981) while progabide exhibits agonist activity at both $GABA_A$ and $GABA_B$ receptors (Bowery et al., 1982). Conversely, Lloyd et al. (1985) have shown that chronic antidepressant treatment (including pargyline, TCA's, 5-HT- or NA-selective re-uptake inhibitors and mianserin), or repeated electroshock, increases the number of $GABA_B$ receptors in the rat frontal cortex but not hippocampus. Cross and Horton (1984), however, have demonstrated that chronic (21 day) treatment with desipramine or zimelidine, at doses which down-regulated 5-HT_2 receptors in the rat frontal cortex, failed to alter the number of $GABA_B$ binding sites in the whole cortex. Thus, the current data indicate that chronic antidepressant treatment may down-regulate $GABA_A$ receptors but, as shown in one study at least, up-regulate $GABA_B$ receptors (thereby modifying the function of GABAergic systems), which, if these effects are shown to be a feature shared by other antidepressant treatments, may be important regarding the mode of action of antidepressants. Furthermore, the ability of GABA-mimetics to down-regulate beta-adrenoceptors, possibly as a

result of increasing NA release, indicates a functional link between central GABAergic and noradrenergic systems. If the ability to down-regulate beta-adrenoceptors is indicative of antidepressant efficacy then GABA agonists may prove to be potential antidepressants (Lloyd et al., 1983).

Finally, many antidepressants exhibit relatively high affinities for histamine H_1 and muscarinic receptors compared to their affinities for other neurotransmitter receptors (see sections 1.4.3.2 and 1.4.4), however, chronic treatment with these compounds does not appear to effect muscarinic receptor density (Peroutka and Snyder, 1980).

1.5 Circadian Rhythms and Depression

1.5.1 Introduction

The revolution of the earth around the sun, and its rotation about its own axis once in every 24 hours, determines the seasonal changes and the day-night variations in light intensity, respectively. Such events produce highly predictable rhythmic changes in the external environment to which living organisms have adapted in order to keep their internal milieu as constant as possible, in accordance with Claude Bernard's concept of homeostasis. Thus, an organisms adaptation to rhythmic changes in the external environment is reflected in rhythmic changes in behavioural, physiological and biochemical systems that follow a similar time schedule to those changes in the environment. Rhythmic changes associated with the 24 hour day-night schedule thus give rise to circadian (Latin : circa = about, diem = day) changes in behavioural, physiological and

biochemical processes of organisms. Virtually all eukaryotic organisms exhibit circadian rhythms in their behaviour, physiology and biochemistry. In addition to circadian rhythms, a wide variety of biological rhythms occur with periods ranging from milliseconds (e.g. electrical activity of the brain) to several years. Rhythms with periods less than 24 hours are termed ultradian (see Hildebrandt, 1988), while those with periods greater than 24 hours are termed infradian (see also definitions for other period lengths given in Cornelissen et al., 1988). In addition, rhythms with frequencies of 1 cycle per 28 days (e.g. menstrual cycle in women) or 1 cycle per year (e.g. hibernation) are termed circalunarand circannual, respectively. This section will focus primarily on circadian rhythms, and, where appropriate, circannual rhythms, in the behaviour and biochemistry of experimental animals, and the relationship of rhythm disorders to depression.

1.5.2 Circadian Rhythms

Richter (1922) was probably the first scientist to publish the results of a systematic study on rodent behaviour that suggested rodents exhibit rhythmic activity when housed under constant environmental conditions. However, it was the study of wild mice (Peromyscus) by Johnson (1926) that led to the conclusion that "the animal has an exceptionally substantial and durable self-winding and self-regulatory physiological clock...." (Johnson, 1939). Little research to identify the physiological mechanism(s) involved in circadian clock generation and control was undertaken until the 1960's, whereupon the investigations of Richter (1965, 1967) opened-up the modern era of research on mammalian neuronal clocks.

The most obvious circadian rhythm which dominates an animals behaviour is the sleep-wake cycle. Sleep is composed of alternating ultradian rhythms of rapid-eye movement (REM) and slow-wave (SW) sleep (Mullen, 1983; Koella, 1984). Even under constant environmental conditions sleep-wake and REM-SW sleep cycles persist, indicating that they are endogenously generated (Weitzman et al., 1979). Sleep-wake cycles in man are typical of circadian rhythms in that when allowed to free-run under constant environmental conditions (e.g. constant light or dark, temperature, humidity) the period of oscillation is approximately, but not exactly, 24 hours (Weitzman et al., 1979; Wever, 1984). Similarly, the sleep-wake cycle of laboratory rodents, as indicated by circadian rhythms of locomotor activity of rats, mice or hamsters, exhibit free-running periods close to 24 hours (e.g. Mitler et al., 1977; Eastman and Rechtschaffen, 1983), although it should be noted that variation in light intensity may modify the period of free-running circadian activity rhythms (Fuller and Edgar, 1986). Other examples of rodent physiological systems which are subject to circadian control include feeding and drinking (Strubbe et al., 1986) and thermoregulation (Eastman and Rechtschaffen, 1983). The occurrence of circadian rhythms in mammalian physiology has been extensively reviewed by Rusak and Zucker (1979).

It is characteristic of free-running circadian rhythms that they synchronize, or entrain, with a phase-relationship to an external time cue (termed "zeitgeber") provided the period of the cue is close to 24 hours (Aschoff et al., 1975). Animal studies have shown that if the phase of the zeitgeber is shifted then the circadian rhythm will re-entrain in order to re-establish the constant phase-angle to

the zeitgeber (Aschoff et al., 1975). The duration of re-entrainment and its direction, i.e. whether the re-entrainment occurs by advancing or delaying the phase-shift of the circadian rhythm, are determined by a number of factors including the degree and direction of phase shift of the zeitgeber and the period of the circadian rhythm being studied. The re-entrainment of circadian rhythms after phase-shift of the zeitgeber has been reviewed by Aschoff et al. (1975). In the natural environment the most potent (and therefore important) zeitgeber for the entrainment of circadian rhythms is the light-dark cycle (Aschoff et al., 1975) since this allows regulation of internal events and externally directed behaviours in order to maximize survival of the species. Indeed, circadian rhythms may remain entrained during constant darkness as long as the normal transition between light and dark is indicated by short photoperiods (Strubbe et al., 1986). Periodic food availability also acts as a strong zeitgeber for rodent activity (Stephan, 1984), however, periodic water availability does not appear to be a potent zeitgeber for circadian activity rhythms in the rat (Mistlberger and Rechtschaffen, 1985). In humans social constraints, rather than the light-dark cycle, predominantly predetermines sleep episodes during the sleep-wake cycle (Wever, 1984).

The generation and maintenance of circadian rhythms is a function of the CNS. Richter (1967), using the free-running activity rhythm of blinded rats as a marker of circadian rhythmicity, found that only lesions of the hypothalamus resulted in a loss of circadian locomotor activity. Subsequently, it was demonstrated that complete lesions of the suprachiasmatic nuclei (SCN) of the hypothalamus induced a

loss of rhythmicity of adrenal corticosterone secretion (Moore and Eichler, 1972), of drinking and locomotor activity (Stephan and Zucker, 1972), and of temperature and sleep rhythms in the rat (Eastman et al., 1984). Partial lesions of the SCN, however, have been observed to result in the generation of weak and variable free-running temperature and sleep rhythms (Eastman et al., 1984), or a shift in the period length of free-running rhythms (Rietveld, 1984). It is now known that the SCN are involved in the generation of circadian rhythmicity of a wide variety of physiological functions (see Rusak and Zucker, 1979). If the SCN is an endogenous pacemaker for circadian rhythms, then the ability of the light-dark cycle to act as a zeitgeber must indicate the coupling of a visual pathway to the endogenous pacemaker; thereby mediating the entraining effects of the zeitgeber. The identification of a direct link between the retina and the SCN via the retinohypothalamic tract in hamsters (Rusak and Boulos, 1981) suggests one neuronal pathway by which light entrainment may be achieved. Direct retinohypothalamic tracts to the SCN have been demonstrated in a number of species (see Moore, 1983). In addition to the retinohypothalamic tract, the SCN also receives projections from the anterior hypothalamus, paraventricular nucleus, lateral hypothalamus, retrochiasmatic hypothalamus, midbrain periaquaductal gray, midbrain raphe nucleus, paraventricular thalamic nucleus. ventral lateral geniculate nucleus, the contralateral SCN, and several nuclei of the tuberal hypothalamus. With the exception of the midbrain raphe nuclei and the ventral lateral geniculate nucleus, projections from the SCN include those areas from which it receives projections and, in addition, the lateral septal nucleus (Rusak and Zucker, 1979; Moore, 1983; Silverman and Pickard, 1983; Steinbusch and

Nieuwenhuys, 1983). The SCN has no long ascending or descending projections, and thus the major processing of the SCN output probably occurs at the hypothalamic level. These examples indicate the potential of the SCN to influence the function of a variety of brain areas. Whether the SCN, which appear to be mutually coupled, independent oscillators (Moore, 1983), are the sole source of the circadian pacemaking machinery is questionable. There is good evidence that complete ablation of both SCN may result in internal desynchronization of circadian rhythms rather than in a complete loss of rhythmicity. For example, Stephan (1984) showed that the activity of rats with SCN lesions, maintained under constant dark, entrained to restricted food availability, and, furthermore, the rats anticipated food access with an increase in activity prior to food availability. When food was freely available, however, activity became arrhythmic. In addition, the studies of Clarke and Coleman (1986) demonstrated the persistence of wheel-running and drinking rhythms in SCN-lesioned rats where periods of food deprivation and ad lib food availability were alternated. These observations indicate the existence of a second, albeit weak, oscillator outside the SCN that may entrain to a zeitgeber such as food availability, but that free-runs in the absence of an entraining signal. Furthermore, the studies of Stephan (1986a, 1986b, 1986c) suggest that light-entrainable and feeding-entrainable rhythms in the rat are under the control of two underlying circadian oscillators that are not functionally independent, but are only weakly coupled. Evidence for multi-oscillators have also been obtained from studies in other species, e.g. squirrel monkey (Moore-Ede et al., 1979). The location and properties of circadian oscillators outside the SCN remain unknown, although Moore and

co-workers have suggested the lateral geniculate nuclei (Johnson et al., 1988; Morin et al., 1988), the lateral hypothalamic nucleus, the retrochiasmatic area and the ventromedial nucleus (Moore, 1982; 1983) as possible candidates in the rat. The current evidence suggests that either the SCN is the primary oscillator within the CNS which drives the secondary oscillators, or the SCN provides a means by which the coupling of independent neuronal oscillators is achieved for circadian rhythm generation (Moore, 1982).

Little is known about the substances that mediate transmission in the SCN. Axons containing vasopressin, vasoactive inestinal polypeptide and somatostatin have been identified in the SCN of the rat, and their distribution suggests that these peptidergic neurons comprise a significant population of SCN interneurons indicative of interconnections between divisions of the SCN (Moore, 1983). Furthermore, significant quantities of 5-HT and substance P have been found in the SCN, while acetylcholine and NA have been shown to be present in the area immediately surrounding the border of the nucleus (Moore, 1983; Silverman and Pickard, 1983).

In mammals, the pineal gland is driven by sympathetic input directly controlled by the SCN, mediated, at least in the Syrian hamster, by beta-adrenoceptors on the pinealocytes (Reiter et al., 1986), and displays a rhythm of indoleamine metabolism that is reflected in the circadian rhythm of 5-HT-N-acetyl transferase activity and melatonin secretion (Klein et al., 1979). Peak enzyme activity and melatonin levels occur, in phase, during the dark period of the light/dark cycle in both diurnal and nocturnal animals (Lewy, 1983; Reiter, 1984). Light immediately suppresses melatonin secretion

(Lewy, 1983). Furthermore, administration of melatonin has been shown to entrain free-running activity rhythms of rats except where the animals had previously received complete lesions of the SCN (Cassone et al., 1986), or partly entrain circadian rhythms of previously disrupted activity induced by gradual increases in the photoperiod (Chesworth et al., 1987). These results suggest that the behavioural effects of melatonin depend on an intact circadian system (including the SCN) and that the action of melatonin may be on the coupling mechanism between oscillators generating circadian locomotor activity rhythms, respectively. In both of these studies the onset of activity entrained to the administration of melatonin, suggesting that nocturnal animals equate melatonin administration with the onset of darkness. The pineal content of 5-HT, 5-HIAA, NA and DA exhibit distinct circadian rhythms (Tang et al., 1985) with peak 5-HT and 5-HIAA levels occurring during the light period, while that of NA or DA occurring during darkness. Similarly, circadian rhythms for NA levels in the SCN, locus coeruleus and dorsal raphe nucleus have been observed in rats housed under 12 hour light/12 hour dark conditions, with peak amine levels occurring at the beginning, middle and end of the light period, respectively (Semba et al., 1984). In the same study, the 5-HT content of both the medial and dorsal raphe nuclei exhibited circadian rhythmicity with peak levels occurring in the middle of the light period. Circadian variation in the CSF content of 5-HT and DA, and their acid metabolites, have also been observed in both the rat (Hutson et al., 1984) and, for 5-HT, the monkey (Taylor et al., 1985). The studies of Lemmer and Berger (1978) also demonstrated diurnal variation in DA, but not NA, levels in rat brain. In the rat, circadian rhythms have also been observed in, for example, the activity of some neurotransmitter

synthesising enzymes, e.g. tryptophan hydroxylase, although equivocal results have been reported (see Redfern and Martin, 1987); in neurotransmitter and release levels, and that of their metabolites, in discrete brain areas (see Kafka, 1987); in MAO activity (Bhaskaran and Radha, 1984); and , lastly, in the content and, in some cases, release of hypothalamic neuropeptides, including bombesin, cholecystokinin, neurotensin, substance P and vasoactive intestinal polypeptide (Nicholson et al., 1983). Furthermore, the circadian activities of some neuropeptides in the pineal gland are thought to induce concomitant modulation of the rate of protein synthesis which is reflected in changes in enzyme activity (Schotman and Bohus, 1983). Radio-ligand binding studies have demonstrated seasonal, circadian and possibly, in the case of beta-adrenoceptors and D_2-DA receptors, ultradian rhythms in the number of receptors for various neurotransmitters in rat brain, e.g. $alpha_2$- and beta-adrenoceptors (Wirz-Justice et al., 1980; Kafka et al., 1981; Krauchi et al., 1984); D_2-DA receptors (Naber et al., 1980); 5-HT_1 and 5-HT_2 receptors, labelled by [^{3}H]-spiperone and [^{3}H]-5-HT, respectively (Bruinink et al., 1983; Wesemann et al., 1983); and muscarinic cholinergic, opiate and benzodiazepine receptors (Kafka et al., 1983). The circadian variation in $alpha_2$- and beta-adrenoceptors in the rat appears to be functionally significant since increased food intake at dawn is associated with increased $alpha_2$-adrenoceptor binding, while the reduction in food intake at dusk is associated with reduced beta-adrenoceptor binding (Krauchi et al., 1984). Redfern and Moser (1985) have demonstrated that the head-twitch response of mice (thought to be mediated by stimulation of 5-HT_2 receptors), but not the 5-HT behavioural syndrome (significant components of which are thought to be

5-HT_1-receptor mediated), induced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), exhibited significant circadian variation with peak response occurring during mid-light. These observations are in agreement with the results of Bruinink et al. (1983) who demonstrated a higher number of 5-HT_2 binding sites during the light-period. The circadian rhythmicity of the 5-HT_2-receptor population is thus approximately 180° out of phase with peak 5-HT release (see above), and the function of the change in 5-HT_2-receptor population may be to dampen the consequences of pre-synaptic oscillations (Redfern and Martin, 1987). Although only a limited number of examples have been provided here, there is ample evidence indicating that the activity and function of the majority, if not all, of the major neurotransmitter systems are under some form of circadian control.

1.5.3 Circadian Rhythm Abnormalities and Depression

Initial clinical evidence implicating circadian or circannual rhythm abnormalities in affective disorders arose from three observations. Firstly, it is generally recognised that external factors which exhibit circadian rhythmicity may alter the mood of depressed patients throughout the day. Thus patients may feel particularly depressed either in the morning after awakening or in the evening (Kripke, 1983). Secondly, the phase-position of the circadian rhythm governing the spontaneous termination of sleep may be shifted abnormally early such that depressed patients experience early wakening. Furthermore, Wehr et al. (1979a) have demonstrated that advancing the sleep-wake cycle of some depressed patients produces a temporary antidepressant effect. Lastly, it has been observed that the degree of depression in some patients follows a seasonal pattern

such that patients feel worse during the winter months than during summer, or vica versa, i.e. Seasonal Affective Disorder (SAD) (see Wehr, 1988). Furthermore, some patients with uni-polar depression exhibit fluctuations between "good" days and "bad" (i.e. depressed) days with clock-like regularity (e.g. 48h depressive cycles; see Doerr et al., 1979). In some cases, bipolar patients exhibit a regular alternation between depressive and manic episodes with a complete cycle period as short as two to eight weeks (Wehr, 1988). Such cases are indicative of rapid cycling forms of affective illness.

Depressive patients exhibit marked differences in their sleep patterns compared to normal individuals. The studies of Borbely et al. (1984) suggest that the development of a sleep-dependent process is deficient in the sleep regulation of depressed patients. The most persistent finding is shortened REM latency which is found in virtually all primary, but not secondary, depressive illness (Kupfer, 1976). A common explanation of these findings is that the circadian rhythm of REM sleep is abnormally advanced with respect to normal sleeping patterns (Wehr and Wirz-Justice, 1982; Kripke, 1983; Mendlewicz et al., 1983; Wehr and Goodwin, 1983b). However, other groups have suggested that the early occurrence of REM sleep may be due to reduced amplitude of the circadian rhythm of REM sleep without phase advance (Schulz and Lund, 1985), or that REM sleep abnormalities in depressives are limited to the initial stages of sleep and decreased SW sleep production is more consistent in depressives than REM sleep abnormalities (Beersma et al., 1983). Furthermore, short REM latency has been observed by some workers to occur in depressed patients that exhibit a small difference between

day and night values of body temperature (Schulz and Lund, 1983), however it is doubtful whether these observations may serve as a marker for depression since similar observations were made in some patients after remission and in some control subjects. Phase-advance of the circadian rhythm of temperature in depressives compared to controls has been observed by some workers (Wehr et al., 1982; Wehr and Goodwin, 1983b), but not by others (Beersma et al., 1983). In addition, Avery et al. (1982) noted that while there was no evidence that the nightly temperature minimum of depressives occurred any earlier compared to control subjects, a number of patients exhibited a delay in the nightly temperature minimum following recovery.

Abnormalities in the circadian rhythms of a number of neurotransmitter (principally indoleamine and catecholamine) systems have also been observed in some depressed patients. For example, Malatino et al. (1981) demonstrated that the circadian rhythm of the plasma tryptophan levels for depressive patients was 180° out of phase compared to that of controls. However, Dam et al. (1984) observed little difference between the circadian variation of plasma tryptophan levels of endogenously depressed patients and controls. Serotonin uptake in blood platelets exhibits circadian rhythmicity (Healy et al., 1986a, 1986b), and abnormalities in the circadian rhythm (Healy et al., 1986a, 1986b) and the seasonal variation (Arora et al., 1984; Egrise et al., 1986) of serotonin uptake in blood platelets of depressed patients compared to controls have been observed. Furthermore, the studies of Healy et al. (1986a, 1986b) suggest that while depressed patients exhibit no circadian rhythmicity in platelet 5-HT uptake, remission from depression is associated with restoration of the normal variation in platelet 5-HT

uptake. Urinary MHPG excretion exhibits circadian rhythmicity, and Wehr et al. (1980) have observed that in some bipolar depressed patients peak urinary MHPG excretion is advanced by at least 3 hours compared to controls. Furthermore, abnormalities in MHPG levels in the urine and plasma of depressed patients tend to normalize with successful antidepressant treatment (Halaris, 1984). Abnormalities in the circadian variation of plasma levels of cAMP, dopamine-beta-hydroxylase, cortisol, melatonin, prolactin and TSH for unipolar and bipolar depressed patients have been observed (Mendlewicz and van Cauter, 1979; Wirz-Justice and Arend, 1979; Markianos and Lykouras, 1981; Lewy, 1983; Mendlewicz et al., 1983; Claustrat et al., 1984; Beck-Friis et al., 1985) which, for cortisol and melatonin at least, may normalize during clinical remission (Wetterberg et al., 1982).

The sensitivity of SAD to changes in season implies that the illness can be precipitated by changes in the physical environment. Furthermore, manipulation of the environment may be of use in the treatment of SAD. Indeed, winter depression has been shown to respond very well to phototherapy using bright light, while heat is strongly implicated in summer depression (Wehr, 1988).

Halberg (1968; cited by Wehr and Goodwin, 1983a) proposed that rapidly cycling episodes of bipolar depression might be caused by abnormal phase-angles of certain circadian rhythms that were no longer entrained to the light-dark cycle, but free-ran; thereby generating differences in frequency, through beat phenomenon, of one cycle every few weeks. In accordance with this hypothesis, Kripke and co-workers (Kripke et al., 1978) described circadian

rhythms of less than 24h period length in patients with rapidly cycling bipolar depression. Lithium has been shown to slow or delay circadian rhythms in both experimental animals (Kripke and Wyborney, 1980) and manic-depressive patients (Kripke et al., 1978; Kripke, 1983). If circadian rhythms in bipolar depressives are either too fast or too phase advanced, then, it has been argued (Kripke et al., 1978; Kripke, 1983), the ability of lithium to slow or delay circadian rhythms may explain its therapeutic efficacy in affective disorders. In a subgroup of bipolar depressive patients, TCA's (e.g. desipramine) have been observed to reduce the duration of both manic and depressive phases, thereby accelerating the cycling rate between mania and depression (Wehr et al., 1979b; Wehr and Goodwin, 1983b). This finding has obvious treatment implications and, furthermore, indicates that rhythms of mood may be modified by antidepressant drugs.

The examples cited above serve to illustrate that circadian rhythm abnormalities in a number of physiological and biochemical parameters may occur in the affective disorders; for more information the interested reader is directed to the reviews by Wehr et al. (1982), Wehr and Goodwin (1983b) and Wehr (1988).

In the opinion of some authors (see Wehr et al., 1982; Kripke, 1983; Wehr and Goodwin, 1983b) a number of circadian rhythms in depressed patients (e.g. REM sleep, temperature, and plasma cortisol and urinary MHPG levels) are either abnormally advanced in relation to sleep position or blunted (i.e. reduced amplitude) compared to those observed in normal subjects. The former being so then advancing the sleep period to an earlier time should correct the

internal phase disturbance. Indeed, some studies have shown that phase-advance of the circadian sleep-wake cycle to re-establish the normal phase-angle of certain circadian rhythms to the sleep-wake cycle, or total or partial sleep deprivation, provide a temporary remission from depression (Wehr et al., 1979a; Wehr and Wirz-Justice, 1982). Furthermore, since antidepressants may normalize the circadian rhythm abnormalities observed in some patients, it has been suggested by some workers (e.g. Wirz-Justice, 1983) that the clinical efficacy of antidepressants may be related to an ability to re-align the phase-position of certain abnormally positioned circadian rhythms.

1.5.4 Antidepressants and Circadian Rhythms in Animal Studies

A number of biochemical and behavioural studies in animals suggest that antidepressant drugs may modify circadian rhythms. Redfern and Martin (1985) showed that chronic treatment with clomipramine and zimelidine, but not imipramine, delayed the time of peak free plasma tryptophan in rats, while imipramine lowered the free tryptophan levels and abolished the normal 24 hour variation in free tryptophan levels. Furthermore, Wirz-Justice et al. (1982) showed that the nocturnal elevation of pineal melatonin, which normally returns to base levels at light onset, persisted into the light period following chronic clorgyline treatment. Similarly, chronic clorgyline treatment has been shown to modify the normal circadian variation in the number of $alpha_1$- and beta-adrenoceptors, muscarinic cholinergic, opiate, benzodiazepine and dopamine receptors (Wirz-Justice et al., 1982). Following clorgyline treatment some or all of the rhythm characteristics (i.e. amplitude, 24h mean number of receptors, and phase) were affected, although

clorgyline commonly delayed the phase-position of binding to alpha$_1$- and beta-adrenoceptors, opiate and benzodiazepine receptors. Similar changes in circadian receptor binding characteristics have been observed following chronic imipramine and fluphenazine (a dopamine antagonist) treatment, while sleep deprivation only delayed the phase-position of binding to alpha$_1$-adrenoceptors but reduced the amplitude of binding to alpha$_1$- and beta-adrenoceptors, benzodiazepine, muscarinic cholinergic and dopamine receptors (Naber et al., 1982; Wirz-Justice, 1983; Wirz-Justice and Wehr, 1983).

In behavioural studies, chronic treatment with imipramine and clorgyline have been demonstrated to slow or dissociate the circadian rest-activity cycle of female hamsters maintained under constant low-red light (Wirz-Justice and Campbell, 1982), while chronic treatment with clorgyline or pargyline have both been demonstrated to delay the activity onset of hamsters entrained to a 14h:10h light-dark cycle (Wehr et al., 1982). Chronic treatment with clorgyline has also been demonstrated to firstly increase the circadian period, activity/rest ratio and total activity of hamsters maintained under constant darkness (Duncan et al., 1986); and secondly, delay activity onset, dissociate activity or induce arrhythmic activity in hamsters maintained under continuous bright light (Duncan et al., 1988). In comparison, however, repeated electroshock had no effect on the free-running circadian locomotor activity rhythms of rats or mice housed under constant environmental conditions (Mitchell et al., 1987). Lastly, Baltzer and Weiskrantz (1975) demonstrated that chronic treatment with imipramine, maprotiline or pargyline facilitated adjustment of entrained locomotor activity of rats to 12h phase-shift in the light-dark

cycle, while non-antidepressant compounds were ineffective. However, an ability to modify circadian rhythms is not necessarily limited to compounds with known or potential clinical antidepressant efficacy. For example, Borsook et al. (1984) reported lengthening as well as shortening of periods in activity and temperature in squirrel monkeys after oral administration of the GABA-ergic compound, valproate, while Rietveld and Wirz-Justice (1986) showed that chronic treatment with either valproate or methamphetamine shortened the free-running period of food intake, drinking activity and wheel-running activity in blinded rats. It should be noted that the ability of MAOI's or valproate to either delay the activity onset of entrained hamsters or modify the free-running circadian activity and temperature rhythms in the squirrel monkey, respectively, is thought to be mediated by the SCN (Kruse, 1988; Borsook et al., 1984, respectively). Conversely, however, methamphetamine has been shown to induce irregular rhythms in the temperature of SCN-lesioned, blinded rats which, prior to methamphetamine administration, exhibited either complete arrhythmicity or severe amplitude reduction, or period shortening following the lesion (Rietveld et al., 1988). Furthermore, the methamphetamine-induced rhythmicity was shown to entrain to food-restriction. Such observations suggest that while the SCN may be the primary target for compounds such as MAOI's, other psychotropic compounds may modify or induce circadian rhythms independant of the SCN. Furthermore, while there is evidence that some antidepressants, or compounds thought to be potentially antidepressant, may modify behavioural or biochemical indices of circadian rhythms in rodents, it should be noted that only a limited number of compounds have been studied. It therefore remains to be shown whether all clinically antidepressant treatments

possess common effects on behavioural or biochemical indices of circadian rhythms and, perhaps most importantly, whether such effects may be equated to the reversal of circadian rhythm abnormalities observed during the remission of depression in some patients.

1.6 Hypotheses on the Aetiology of Depression

1.6.1 The Classical Monoamine Hypothesis

The classical monoamine hypothesis of depression states that depression is caused by an absolute or relative deficiency of 5-HT (Coppen, 1967) and/or NA (Schildkraut and Kety, 1967) available for neurotransmission within the CNS. The hypothesis was derived primarily from studies on the acute pharmacological effects elicited by the majority of clinically effective MAOI's and TCA's available during the 1960's and 1970's. In addition, the antidepressant efficacy of the serotonin precursors, tryptophan and 5-HTP (see review by van Praag, 1981b), together with clinical studies on the concentrations of monoamines and their metabolites in the CSF or post-mortem brain samples (e.g. Ashcroft et al., 1966) supports this view. There are two major, equally important, criticisms of the hypothesis. Firstly, the hypothesis is primarily limited to a consideration of the drug-induced modification of pre-synaptic neuronal function, and thus effectively ignores any indirect drug-induced modification of the post-synaptic signal tranduction mechanisms. Secondly, the hypothesis does not accommodate the discrepancy in the time course between the biochemical and pharmacological effects elicited by antidepressant drugs and their clinical therapeutic action which generally requires several weeks of

continuous treatment (Oswald et al., 1972). Furthermore, the pharmacological profile of the atypical antidepressants mianserin and, more especially, iprindole, together with the lack of antidepressant efficacy of the NA re-uptake inhibitor cocaine, suggests that an ability to potentiate monoamine function, either by inhibition of 5-HT or NA re-uptake or MAO activity following acute treatment, is not necessarily a prerequisite for antidepressant activity. In addition, the 5-HT_2 receptor blocking activity of mianserin is not in accord with the classical monoamine deficiency theory of depression. While it would not be denied that increased monoamine function resulting from antidepressant treatment is probably inherent in the initiation of the therapeutic efficacy of the majority of antidepressants, such effects do not necessarily indicate a deficiency of 5-HT or NA in the pathogenesis of depression.

1.6.2 The Alternative Monoamine Hypothesis

Studies on the delayed post-synaptic receptor mediated events induced by chronic antidepressant treatment in rodents have led to an alternative monoamine hypothesis for the pathogenesis of depression that is in closer accord with the clinical latency of these compounds. Generally, chronic antidepressant treatment, including electroshock, leads to down-regulation of beta-adrenoceptor number and hence reduced sensitivity of the NA-dependent adenylate cyclase system (see section 1.4.5), while a persistent reduction in central NA availability leads to increased sensitivity of the cAMP generating system (Vetulani et al., 1976b). These observations led to the hypothesis, initially proposed by Vetulani, Sulser and colleagues (see Vetulani et al., 1976a; Sulser et al., 1978), that in

depression there may be a post-synaptic supersensitivity to NA and that antidepressant treatments, including ECT, bring about a desensitization of enhanced noradrenergic function and hence a return to behavioural normality. In this context the low levels of monoamine metabolites observed in the CSF of some depressed patients prior to antidepressant treatment may be due to a compensatory decrease in monoamine biosynthesis rather than directly related to the pathogenesis of depression. This hypothesis has been extended to include the observed down-regulation of both the $alpha_2$ receptor-mediated negative feedback system (which may be necessary for the succeeding down-regulation of beta-adrenoceptors) and central post-synaptic 5-HT_2 receptors. Although there are exceptions, the aforementioned changes in receptor density, and their consequences for signal transduction, normally only follow chronic antidepressant treatment. The hypothesis therefore accommodates not only the known pharmacological and biochemical effects observed following chronic treatment with the atypical antidepressants, mianserin and iprindole, but also the latency of effect required for remission from depression experienced in the clinic.

1.6.3 The Circadian Rhythm Phase-Advance Hypothesis

It has been suggested by some workers that certain forms of depression may be produced following a disruption or de-synchronization of circadian rhythms (Wehr and Wirz-Justice, 1982; Wehr et al., 1982; Kripke, 1983). The hypothesis is based on the observation that the phase-position of the circadian rhythm governing the spontaneous termination of sleep may be shifted abnormally early; thus depressed patients often experience early wakening. Furthermore, Wehr et al. (1979a) have shown that

advancing the sleep-wake cycle of some depressed patients produces a temporary antidepressant effect. Wehr and Wirz-Justice (1982) have therefore proposed that the phase-position of certain circadian rhythms are advanced in relation to other circadian rhythms in some depressed patients, and the resultant modified phase-relationship between circadian rhythms is manifest as depression. In addition, it has been argued that the free-running hypothesis of Halberg (see section 1.6.2), to explain the rapid-cycling of manic-depressive episodes in some bipolar patients, may be viewed as an extreme development of the phase-advance hypothesis of depression (see Kripke, 1983; Wehr and Goodwin, 1983a). Likewise, it has been suggested that SAD may be due to phase-shift of circadian rhythms relative to the timing of sleep (see Wehr, 1988, for discussion). It therefore follows that if phase advances in circadian rhythms were a primary pathophysiologic mechanism of depression then antidepressants may exert their therapeutic effect by reversing excessive phase advance.

1.7 Identification of Potential Antidepressants

The aim of this section is to briefly summarize the pharmacological, behavioural and biochemical techniques employed to identify potential antidepressants or elucidate the pathophysiological processes of depression.

1.7.1 Animal Models of Depression

Animal models of depression may be divided into two general categories.

Firstly, many models have been developed for the purpose of identifying novel compounds of potential clinical antidepressant efficacy. These tests include reversal or protection of the behavioural effects of monoamine depletion (following treatment with reserpine, tetrabenazine, 6-OHDA or pCA) or monoamine synthesis inhibition (by pCPA or alpha-methyl-para-tyrosine); potentiation of directly or indirectly acting sympathomimetics (e.g. L-DOPA plus a MAOI, amphetamine) either centrally or peripherally; potentiation of the lethal effects of the $alpha_2$-antagonist, yohimbine, in mice; potentiation of the behavioural syndrome induced by serotonin precursor loading (e.g. 5-HTP or tryptophan plus a MAOI); inhibition of muricide behaviour (rats); inhibition of seizure activity elicited from the amygdala, rather than the cortex, in rats induced by low-intensity electrical stimulation (known as kindling); reversal of the behavioural effects of olfactory bulbectomy (rats); and inhibition of isolation-induced hyperactivity in rats (Moller Nielsen, 1980; Katz, 1981; Willner, 1984). It should be noted that each test makes no assumptions about the relationship of the test procedure to the underlying mechanisms of depression nor about such mechanisms per se. In each case the tests are employed because of their empirical utility and thus only identify "me-too" compounds. Furthermore, each test progressively loses its power to detect novel compounds as the chemical structure of the latter progressively differs from that of the more traditional antidepressants upon whose detection the rationale for each test was based. In addition, the empirical power of each test varies and the potency order of compounds identified by a particular test may not follow that observed in the clinic (Willner, 1984). A further criticism of these tests is that each has the ability to identify false positives

(i.e. compounds proving positive in the test but which have no antidepressant efficacy). For example, the effects of reserpine may be reversed by L-DOPA, amphetamine, histamine antagonists, LSD and beta-adrenoceptor antagonists, but mianserin is not detected by this test; muricide may be blocked by psychostimulants, antihistamines and anticholinergics, but not by mianserin; MAOI's do not normalize the behaviour of olfactory bulbectomized rats (Cairncross et al., 1979); and, lastly, tests which employ MAOI's as adjuvants to monoamine precursor loading obviously cannot identify novel MAOI's (Willner, 1984). Perhaps the most important criticism of these tests is that they identify potential antidepressant compounds following acute treatment, and thus effectively ignore the relevance of chronic treatment regimes which are required in the clinic. It may be claimed that such tests have been successful in identifying potential "me-too" antidepressants, however it may also be argued that reliance on such tests has effectively hindered the identification and development of potential antidepressants of novel structure and mode of action. The only animal behaviour that consistently and clearly responds differently to acute or chronic antidepressant treatment is aggressive behaviour (see review by File and Tucker, 1986). Thus antidepressants, among other classes of psychotropic compounds, reduce various types of rodent aggressive behaviour following acute treatment (e.g. Horovitz et al., 1966; Crowley, 1972; Malick, 1976; Sheard et al., 1977; Gibbons et al., 1978b; Delini-Stula and Vassout, 1979), while only chronic treatment regimes clearly identify examples of antidepressants as shown by the drug-induced increase in aggressive behaviour (e.g. Eichelman and Barchas, 1975; Mogilnicka and Przewlocka, 1981;

Willner et al., 1981; Prasad and Sheard, 1982; Valdman and Poshivalov, 1986).

The second class of animalmodel has been concerned with the theoretical basis of depressed behaviour rather than with drug screening and development. Such models include learned helplessness, where chronic exposure to uncontrollable stress produces performance deficits in subsequent learning tasks, decreased locomotion and aggression, and loss of appetite and weight (see reviews by Katz, 1981; Weiss et al., 1982; and Willner, 1984); behavioural despair, where mice or rats assume an immobile posture following a frenzied attempt to escape on being forced to swim in a confined space (Porsolt et al., 1977); chronic unpredictable stress, where rats are chronically exposed to number of mild unpredictable stressors (see Willner et al., 1987, for details); maternal or peer separation models in a number of species (Katz, 1981), including non-human primates, where the initial manifestations of protest are soon followed by those of despair (Willner, 1984); and intracranial self-stimulation (ICSS; see Willner, 1984, File and Tucker, 1986). Chronic, but not acute, antidepressant treatment generally reduces the performance or behavioural deficits observed in the learned helplessness, chronic unpredictable stress and separation models (Willner, 1984; File and Tucker, 1986), thus demonstrating accordance with the clinical latency of these compounds. However, the theoretical validity of these models to endogenous depression has been questioned (Willner, 1984). The behavioural despair test may be criticized on the basis that the period of immobility during forced swimming is demonstrably sensitive to acute antidepressant treatment, including single electroshock,

and, furthermore, the test may identify false positives such as amphetamine and caffeine (Porsolt et al., 1977). The tail-suspension test, where immobility is also taken as an index of behavioural despair (Steru et al., 1985) may also be similarly criticized, although the generalised predictive value of the behavioural despair would not be denied. ICSS may be criticized on three points. Firstly, there is no consistent single effect of antidepressants on brain-stimulation reward (thus some compounds enhance self-stimulation, especially those that may facilitate dopaminergic transmission, while others decrease the reward value of electrical stimulation); secondly, the antidepressant-induced modification of ICSS depends largely on electrode placement; and lastly, no clear differences can be discerned between acute and chronic treatment regimes (File and Tucker, 1986).

It may be argued that animal models utilizing biological rhythm disturbance have a theoretical basis since they attempt to mirror the circadian rhythm disturbances observed in some depressed patients. Such models fall into two categories. Firstly, compounds are tested for their ability to modify the adjustment of activity patterns exhibited by animals to phase-shifts in the daily light-dark cycles. Chronic treatment with some antidepressants has been demonstrated to facilitate adjustment to phase-shift, while non-antidepressant compounds were ineffective (Baltzer and Weiskrantz, 1975). Secondly, compounds have been tested for their ability to modify the free-running activity rhythms of animals. In this case, chronic treatment with imipramine or clorgyline to female hamsters, housed under constant environmental conditions, has been found to slow or dissociate free-running circadian locomotor activity rhythms

(Wirz-Justice and Campbell, 1982). The effect of antidepressants on circadian rhythms has received scant attention in the literature, and it is not known whether all antidepressant treatments, including electroshock, modify circadian rhythms in a similar manner to the examples given above. Furthermore, it is unlikely that the theoretical basis of the studies described above is justified. Thus, in both cases cited above the phase position of the particular circadian rhythm monitored is assumed to be in its normal position in relation to other circadian rhythms; indeed circadian rhythms may become desynchronized during chronic antidepressant treatment (Wirz-Justice and Campbell, 1982). Conversely, the abnormal phase position of some circadian rhythms in depressed patients prior to drug treatment are thought to be re-synchronized following drug treatment. It is therefore unlikely that the studies cited above mirror the circadian rhythm disturbances in depression. These studies may therefore only prove to be of empirical interest.

1.7.2 Biochemical Methods

Biochemical studies employed in antidepressant research may be divided into two categories.

The first category is comprised of those studies primarily concerned with pre-synaptic neuronal function. Such studies include the ability of antidepressants to modify neurotransmitter uptake, either *in vitro* or *in vivo*, and simply demonstrate selectivity for a particular neurotransmitter re-uptake system. The ability of antidepressants to protect against monoamine depletion, induced by, for example, reserpine, tetrabenazine or pCA, only enable similar conclusions. The effect of acute antidepressant treatment on the

levels of monoamines or their metabolites, or the activity of the enzymes involved therein, have only demonstrated the ability of antidepressants to modify pre-synaptic neuronal function. Furthermore, similar studies involving chronic antidepressant treatment have generally only demonstrated the ability of neurotransmitter systems to modify their activity according to changes in the synaptic or intraneuronal concentration of the respective neurotransmitter. Specific high-affinity binding sites for [^{3}H]-imipramine have been described in the brain and platelets of several mammalian species, including man (see review by Briley, 1985). The binding sites for [^{3}H]-imipramine are located on serotonin nerve terminals and appear to be related to, but not identical with, the transporter mechanism that translocates 5-HT through the neuronal and platelet membrane (Langer and Raisman, 1983). The [^{3}H]-imipramine binding site appears to be a regulatory unit capable of modulating 5-HT re-uptake (Briley, 1985). Similarly, [^{3}H]-desipramine binds to high affinity sites (Biegon and Samuel, 1979) thought to be associated with NA re-uptake in the brain (Rehavi et al., 1981, 1982) and periphery (Langer et al., 1981). A number of TCA's bind directly to the [^{3}H]-imipramine site (e.g. clomipramine, amitriptyline, desipramine) while 5-HT and non-tricyclic inhibitors of 5-HT re-uptake appear to bind to the 5-HT recognition site of the re-uptake complex and inhibit [^{3}H]-imipramine binding by an indirect mechanism (Briley, 1985). Binding to the [^{3}H]-imipramine site has been used as a method of examining the interaction of putative antidepressants with the 5-HT re-uptake system. Such studies have undoubtedly led to a greater understanding of monoamine re-uptake systems, however, their use as screening tests for potential antidepressants is largely limited to empirical

value. Similar conclusions may only be drawn from similar studies examining the binding sites labelled by other radio-labelled monoamine re-uptake inhibitors.

The second category of biochemical tests is comprised of those experimental techniques concerned primarily with post-synaptic function and include such tests which identify neurotransmitter binding sites and the activity of second messenger systems. Such studies have demonstrated that a number of antidepressants exhibit comparatively high affinities for some neurotransmitter receptors, and have provided possible explanations for a number of side-effects associated with some antidepressant treatments. It is only the effect of chronic antidepressant treatment on the binding characteristics of some radio-ligands, or the activity of some second messenger systems (particularly NA-dependent adenylate cyclase) that has enabled a greater understanding of the mechanisms by which antidepressants may induce their clinical effect. Even so, such studies make no assumptions about biochemical deficits that may be associated with depression.

In circadian rhythm research biochemical studies have concentrated on the expression of rhythmicity determined by the above techniques and their modification by antidepressant treatments. As such, these studies probably represent the only biochemical studies with a theoretical relationship to the possible circadian rhythm abnormalities observed in some depressed patients. However, since such studies are concerned primarily with circadian rhythms in the biochemistry of normal animals, they are open to the same criticisms as the animal models, and ultimately therefore, may only prove to

be of empirical value.

1.7.3 Comments

The majority of pharmacological, behavioural and biochemical tests used to study antidepressant drug action, or identify and develop potential antidepressants, have generally been derived from the previously determined mode of action of available antidepressants, or the ability of available antidepressants to modify abnormal animal behaviours or biochemistry induced by pharmacological tools which result in behavioural or biochemical deficits assumed to be consistent with a single facet of the pathology of depression. As such, these tests are primarily of empirical utility. Consequently, their power is limited to the identification of novel compounds whose primary mode of action is akin to those compounds already available to the clinician. Even when those animal models with a theoretical relationship to depression are considered there does not appear to be a definitive model of depression, and this largely reflects the level of understanding of the pathophysiology of the affective disorders. One facet of clinical depression is decreased interest in sexual and social activity (see section 1.2.4.1), and these emotional and behavioural changes are reversed by antidepressant treatment. In this respect it is disappointing that scientists have largely ignored the intrinsic effects of chronic antidepressant treatment on endogenous patterns of normal animal behaviour, but have instead concentrated on the ability of antidepressants to modify abnormal animal behaviours induced either by pharmacological tools or exotic experimental paradigms. As File and Tucker (1986) noted in their review on the effects of antidepressants on animal models of behaviours that are changed during clinical depression: "In general,

the only type of social behaviour that is increased by antidepressant treatment is aggression in the rat. This effect is particularly marked for antidepressants that alter noradrenergic transmission and only occurs after chronic treatment." There are two important points to note here. Firstly, the increased expression of rodent aggressive behaviour induced by antidepressants may be consistent with the reversal of human aggressive behaviour from intropunitive hostility to extrapunitive hostility following clinical antidepressant treatment (Priest et al., 1980); and secondly, such behavioural effects were only observed after chronic antidepressant treatment, which is consistent with the clinical latency of these compounds.

1.8 Conclusions

The symptomology of depressive illness has been well described and a number of rating scales have been developed by which the clinician may gauge both the severity of the illness and the response to antidepressant therapy. Clinical studies suggest that 2-3 weeks of continuous treatment is required before any remission from depression is observed.

Biochemical studies in depressed patients, together with the identification of the acute and chronic effects of antidepressant drug administration on central serotonergic and noradrenergic function in experimental animals, suggest that depression is associated with abnormalities in the function of central serotonergic and noradrenergic neurotransmitter systems. Such neurotransmitter abnormalities are also implicated in some circadian rhythm abnormalities, observed in a limited number of depressed patients,

that may play a causative role in the symptomology of some depressive states. However, while such studies have led to three principal hypotheses on the aetiology of depression, the underlying pathophysiology of the disease state remains to be elucidated. It is unlikely that depression is a unitary disease and, consequently, the probability that a single pathophysiology underlies the various forms of depressive illness is small.

The majority of antidepressant drugs have been identified on the basis of their acute mode of action, thereby ignoring the clinical latency of antidepressant treatment. Furthermore, the methods currently employed to identify potential antidepressants are largely of empirical utility and thus make no assumptions about their relationship to the mechanisms that play a role in depression. It would not be denied, however, that the currently available antidepressant drugs have shown a high level of success, especially when one considers that the majority of depressed patients are able to remain within the community during treatment. Such patients are therefore exposed to the same environmental and social stimuli, which prior to therapy they found intolerable, throughout the duration of their illness. This indicates that the patients' response to environmental and social stimuli must change with chronic antidepressant treatment. Clinical studies have suggested that the remission from depression is associated with a reduction in the intrapunitive manifestations (e.g. guilt) of the disease which, it is suggested, may also involve a drug-induced readjustment of abnormally positioned circadian rhythms. The possible involvement of circadian rhythm abnormalities in depression is a relatively new approach, and it remains to be seen whether an ability to modify

circadian rhythms is common to all antidepressant treatments.

Although a high level of commonality has been identified in the biochemical effects following chronic treatment with antidepressants in experimental animals, it is surprising that researchers have largely ignored the logical extension of the common ability of antidepressant treatments, including ECT, to modify the reactive patterns of human behaviour, concomitant with the remission from depression, to studies on the ability of antidepressant treatments to modify the normal behavioural patterns of experimental animals.

CHAPTER 2 AIMS OF THIS STUDY.

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If antidepressants have the ability to modify either reactive behaviour patterns or the relative phase position of circadian rhythms of depressed patients then it is reasonable to assume that either the normal social behaviour patterns or endogenous circadian rhythms of laboratory animals may also be modified following chronic treatment with known antidepressant agents. This project is therefore based on the hypothesis that the efficacy of antidepressants is related to an ability to alter endogenous patterns of rodent behaviour in terms of either social behaviour or circadian locomotor activity rhythms.

The effects of acute and chronic treatment with clinically established antidepressants on the behavioural profile exhibited by resident rats during social interaction with unknown intruder conspecifics have been evaluated. This behavioural model maximizes the level of physical contact between subjects, thereby allowing the maximum expression of rodent social behaviours which may be observed and recorded. The antidepressants used in this study were chosen as representatives of the major classes as indicated by their acute pharmacology, i.e. the tricyclic antidepressant clomipramine, the MAOI phenelzine and the "atypical" antidepressants iprindole and mianserin. In addition, the effects of similar treatment regimes with the potential antidepressant fluoxetine, which specifically inhibits the re-uptake of 5-HT, and, for comparison, the antipsychotic haloperidol and the anxiolytic diazepam, on this animal model of rodent social behaviour have also been examined.

The effects of acute and chronic administration of each drug on the exploratory locomotor activity of rats were examined to establish whether any of the observed drug effects on social behaviour could be attributed to drug-induced changes in the basal level of rodent activity.

The effects of chronic clomipramine and mianserin treatment on the rank position of subdominant rats were examined to establish whether the effect of similar drug treatment regimes on social behaviour might be indicative of drug-induced changes in social drive.

Finally, the effects of chronic clomipramine, fluoxetine and mianserin, administered at clinically-equivalent doses, on the free-running circadian locomotor activity of rats housed singly or in groups have been examined.

In summary, the purpose of these experiments has been to test the hypothesis that a feature common to the chemically-disparate group of drugs clinically labelled "antidepressant" is an ability to modify the various elements of social and aggressive behaviour and circadian rhythms.

CHAPTER 3 OVERVIEW OF THE RATIONALE OF EXPERIMENTAL PROTOCOLS

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The aim of this chapter is to provide a short introduction to the different series of experiments described in the following chapters together with the rationale supporting both the choice of experimental methodology and the various drugs examined.

3.1 Social behaviour

The primary objective of these studies was to examine the effect of chronic antidepressant treatment on rodent social behaviour. The method of quantifying rodent social behaviour was based on the social interaction test described by Silverman (1965). This method records the frequency of the repertoire of postures or behaviours exhibited by the subject animals, thus providing an accurate, quantifiable profile of rodent social behaviour.

Initial experiments examined the effects of acute, ascending doses of each drug in order to identify the minimum behaviourally-active dose of each compound which, in a separate series of experiments, was administered continuously for 14 days, by subcutaneously-implanted osmotic mini-pumps. This method of chronic administration was chosen since constant drug delivery results in constant plasma/tissue levels of the drug, as opposed to the peak effects associated with bolus injection, and obviated the need for excessive handling of the subjects and thus reduced injection-associated stress.

The effects of acute and chronic drug administration on the exploratory locomotor activity of rats were examined concomitantly with the social interaction studies. Such studies were designed to

identify whether any drug-induced effects on rodent social behaviour might result from drug-induced changes in basal activity rather than specific effects on social behaviour.

Lastly, the effect of chronic antidepressant treatment on the rank position of subdominant rats maintained in social groups was examined to determine whether the effects of similar treatment regimes on the endogenous patterns of social behaviour might be indicative of drug-induced changes in social drive.

A detailed description of each experimental method is provided in the relevant chapter.

In all these experiments drugs, whether administered acutely or chronically, were given by the subcutaneous route since the extent to which absorption occurs via the hepatic portal system (with a first pass through the liver), as opposed to the systemic circulation, is uncertain following intraperitoneal administration, whether by injection or osmotic mini-pump. For substances that are extensively metabolized by the liver the intraperitoneal route may produce highly variable concentrations of the drug in the systemic circulation and therefore variable pharmacological/behavioural effects. Similarly, drugs absorbed via the gastric or intestinal mucosa following oral administration are also subject to the possibility of first-pass metabolism following removal of the drug to the hepatic circulation. The subcutaneous route of drug administration was therefore chosen for these experiments, although the rate of absorption may be slower than that observed following administration via alternative routes.

3.2 Circadian locomotor activity

The objective of these studies was to examine the effect of chronic antidepressant treatment on the "free-running" circadian locomotor activity rhythms of rats. All experiments were performed with the subject animals housed in environmental cabinets to maintain constant environmental conditions as required by the experimenter.

The plethora of data generated by these experiments consists of continuously sampled counts of activity accumulated at discrete time points throughout the duration of the experiment, and are indicative of complex waveforms containing cycles of activity that differ in their respective frequency, amplitude and phase position. Such data may be subjected to Time-Series analysis to identify the component frequencies (and amplitude) of activity. The accuracy of the statistical methods, however, is directly related to the number of fundamental cycles contained within the data sample analysed. The duration of each experiment was therefore determined not only by the required duration of drug treatment but also by the requirements of the statistical methods employed. The accuracy of the statistical methods is also reduced with the occurrence of missing data points or transiently high levels of activity that may occur, for example, following handling of the animals for drug administration or necessary husbandry. All experiments were therefore largely self-contained such that the subject animals were supplied with sufficient food and water to last at least 7 days (and longer if possible) thereby reducing interference with the animals to a minimum during the course of each experiment. For the same reason, animals were treated chronically with drugs or vehicle presented in the drinking water rather than by subcutaneous administration as employed

in the social behaviour studies.

3.3 Choice of drugs

The antidepressants examined in these studies were chosen as representatives of the clinically-established tricyclic (clomipramine), mono-amine oxidase inhibitor (phenelzine) and "atypical" (iprindole and mianserin) classes of antidepressants. The effects of fluoxetine, a potential antidepressant currently under development, were also examined. For comparison, the effects on rodent social behaviour of the antipsychotic haloperidol and the anxiolytic diazepam were examined. The chemical structures of these compounds are depicted in Fig. 3.1.

The following sections provide a brief resume of the background information pertinent to each of the antidepressants examined in the following studies. A review of the pharmacological actions of haloperidol and diazepam will not be provided here; for further information the reader is directed to any standard neuropharmacology textbook.

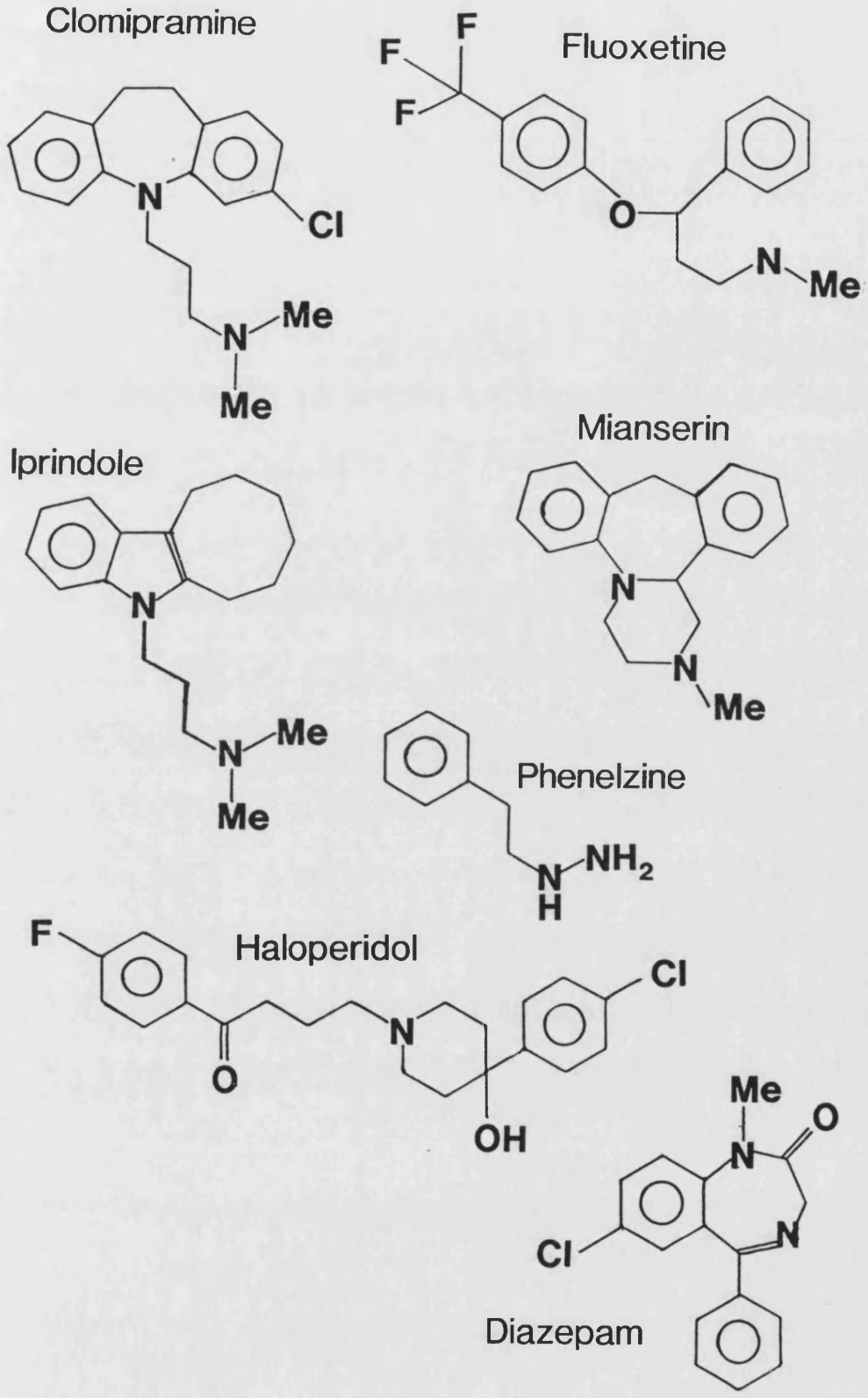


Fig. 3.1 Chemical structures

3.3.1 Clomipramine

During clinical investigation of a series of phenothiazine analogues for sedative or hypnotic properties, Kuhn (1958) observed that imipramine, a compound originally developed as an anti-histamine, was relatively ineffective in quieting psychotic patients but showed beneficial effects when administered to certain depressed patients. This observation led to the search for other chemically-related "tricyclic" compounds that also possessed antidepressant activity. One of these compounds was the 3-chloro derivative of imipramine, called clomipramine. The metabolism of clomipramine resembles that of the parent compound imipramine. Thus the major metabolites are the mono-demethylated derivative (which, as for imipramine, is likely to participate in the therapeutic action of clomipramine) and several hydroxylated compounds (Bickel, 1980, and references cited therein).

It is now widely accepted that one important mechanism of action of the tricyclic antidepressants is the ability to inhibit the high-affinity, energy requiring, re-uptake systems of NA and/or 5-HT (Carlsson, 1970). In this respect clomipramine, and its des-methyl metabolite, are approximately equipotent on 5-HT and NA re-uptake with comparatively little or no significant effect on the re-uptake of DA (Sulser and Mobley, 1980).

In radio-ligand binding studies clomipramine exhibits marked affinity for histamine H_1 and $alpha_1$-NA receptors (see reviews by Green and Nutt, 1983; and Leysen, 1985).

Clomipramine, originally called chlorimipramine, was first used

clinically in 1964 by Brandner (cited by Waxman, 1977) and since then it has become firmly established among the repertoire of antidepressants available to the clinician. The British National Formulary (BNF) recommends that initial treatment with clomipramine, presented as the hydrochloride salt, starts at 10 mg (by mouth) daily and the dose be increased gradually to the maintenance dose of 30-150 mg or more in severe depression and phobic and obsessional states, although Paykel (1979b) suggests that the usual daily dose range is 75-200 mg.

Clomipramine was chosen for examination in these studies on the basis of its typical tricyclic antidepressant profile (see sections 1.4.3 and 1.4.5, and references cited therein).

3.3.2 Fluoxetine

Fluoxetine is a new antidepressant which differs structually and pharmacologically from the tricyclic compounds. Fluoxetine has been reported to inhibit specifically the re-uptake of 5-HT into synaptosomes of rat brain (Wong et al., 1974) and in specific brain regions in vivo (Wong et al., 1975), with comparatively little effect on NA re-uptake in vitro (see also Harms, 1983; Iversen and Mackay, 1979, Waldmeier et al., 1976). Like other 5-HT re-uptake inhibitors, fluoxetine reduces the level of 5HIAA without affecting the overall level of 5-HT (Perry and Fuller, 1974) and also reduces the firing rate of raphe neurons (Clemens et al., 1977), indicating that fluoxetine reduces 5-HT turnover, presumably through a negative feedback system. Unlike clomipramine or imipramine, N-demethylation of fluoxetine does not alter either its potency or selectivity to inhibit 5-HT re-uptake (Wong et al., 1975).

In in vitro radio-ligand binding studies fluoxetine demonstrated low affinity for alpha- or beta-adrenoceptors, histamine H_1, muscarinic acetylcholine, 5-HT, opiate, GABA, benzodiazepine or DA receptors (Peroutka and Snyder, 1980; Wong et al., 1983). In one study chronic treatment with fluoxetine, 10 mg Kg^{-1} i.p. daily, reduced the number of 5-HT_1 receptors in rat cerebral cortex (Wong et al., 1985), but this effect was not observed in the earlier study by Peroutka and Snyder (1980).

The down-regulation of beta-adrenoceptors and the subsensitivity of NA-stimulated adenylate cyclase induced by chronic treatment with tricyclic antidepressants have both been suggested as models of the time-dependent antidepressive response to antidepressants in humans (Banerjee et al., 1977; Vetulani et al., 1976a; Sulser et al., 1978). Chronic treatment with fluoxetine, however, failed to down-regulate cortical beta-adrenoceptors (Wong et al., 1985) or to reduce the sensitivity of NA-stimulated adenylate cyclase (Schmidt and Thornberry, 1977).

The selectivity of fluoxetine for inhibition of 5-HT re-uptake in vivo has recently been questioned. Fletcher et al. (1988) demonstrated that acute treatment with fluoxetine at doses within the range required for 5-HT re-uptake inhibition also reduced tetrabenazine-induced ptosis (indicative of catecholamine re-uptake inhibition) and potentiated and prolonged amphetamine-induced stereotypy (indicative of increased central dopaminergic activity). The involvement of central catecholamine systems, in addition to 5-HT, in the mediation of the antidepressant effects of fluoxetine cannot therefore be discounted.

In double-blind clinical trials fluoxetine, mean doses 55-60 mg day^{-1}, demonstrated similar or better antidepressant efficacy than 175-200 mg day^{-1} imipramine (Bremner, 1984) or 145-173 mg day^{-1} amitriptyline (Feighner, 1985). Both reports indicated a lower incidence of side effects with fluoxetine than with either imipramine or amitriptyline which may reflect the 5-HT selectivity of fluoxetine. The review by Benfield et al. (1986) suggests a daily dose of 20 mg should be adequate for most patients with a maximum daily dose of 80 mg.
Fluoxetine was chosen for examination in these studies on the basis of its reported 5-HT specificity.

3.3.3 Iprindole

Iprindole is an example of those atypical antidepressants that have little in common with more conventional compounds. Iprindole appears to have no effect on 5-HT, NA or DA uptake either *in vitro* or *in vivo*, neither does it alter NA turnover when given chronically (Rosloff and Davis, 1974; Sulser and Mobley, 1980). Consequently iprindole does not possess the monoamine potentiating effects associated with the tricyclic antidepressants.

In *in vitro* radio-ligand binding studies iprindole demonstrates little or no affinity for $alpha_1$-, $alpha_2$- or beta-adrenoceptors, histamine H_1, muscarinic acetylcholine, 5-HT or DA receptors (Green and Nutt, 1983; Peroutka and Snyder, 1980).

Chronic treatment with iprindole however, like desipramine, induces down-regulation of beta-adrenoceptors (Banerjee et al., 1977; Peroutka and Snyder, 1980) and $5\text{-}HT_2$ receptors (Peroutka and Snyder,

1980), and reduces the sensitivity of the NA-sensitive adenylate cyclase system (Vetulani et al., 1976a). Thus, while the chronic effects of iprindole are in accord with those observed for the more conventional tricyclic antidepressants, an acute mode of action has yet to be elucidated.

Initial clinical trials indicated patients treated with iprindole, up to 90 mg daily (Hicks, 1965) and 30-80 mg daily (Daneman, 1967) showed significant improvement compared to patients receiving placebo. In a further study, Sterlin et al. (1968) reported iprindole, 45-120 mg daily, to be slightly faster acting, but somewhat less effective, compared to amitriptyline, 75-200 mg daily. Initial treatment with iprindole, presented as the hydrochloride salt, starts at 15-30 mg (by mouth) 3 times daily and the dose increased gradually as necessary to a maximum of 60 mg 3 times daily, with the usual maintenance dose being 30 mg 3 times daily (BNF). Paykel (1979b) suggests that the usual daily dose range is 45-180 mg.

Iprindole was chosen for examination in these studies as an example of an atypical antidepressant with a novel mode of action.

3.3.4 Mianserin

The tetracyclic piperazine-azepine mianserin was originally developed during the early 1970's in the search for potent antiserotonin and antihistamine compounds. Its potential use as an antidepressant was predicted on the basis that mianserin produced changes in the human electroencephalogram similar to those produced by tricyclic antidepressants (see reviews by Itil and Soldatos, 1980; Itil,

1983). A number of clinical trials have confirmed the predicted antidepressant potential of mianserin. For example, mianserin, 60 mg daily, was shown to be of similar antidepressant efficacy to 150 mg daily treatment with amitriptyline (Coppen et al., 1976; Vogel et al., 1976; Jaskari et al., 1977), clomipramine (Blaha et al., 1980; De Buck, 1980; Pinder et al., 1980), imipramine (Murphy, 1975; Pichot et al., 1978; Pull et al., 1980) or maprotiline (Khan and Moslehuddin, 1980). In these studies patients receiving mianserin complained of less side effects (especially those associated with anticholinergic activity) than those receiving amitriptyline, clomipramine or imipramine. It has also been claimed that mianserin possesses anxiolytic activity. In a study by Murphy (1978) no significant differences in anxiolytic effect or in side effects were observed between mianserin, 30 mg daily, or diazepam, 15 mg daily.

Initial treatment with mianserin, presented as the hydrochloride salt, starts at 30-40 mg (by mouth) in divided doses and increased gradually as necessary to a maximum of 200 mg daily in divided doses, with the usual maintenance dose range of 30-90 mg (BNF). Paykel (1979) suggests a slightly larger daily dose range of 20-120 mg.

In comparison to the tricyclic antidepressants, mianserin is only weakly or moderately active as an inhibitor of 5-HT or NA uptake *in vitro* respectively (Baumann and Maitre, 1977), and is essentially devoid of effect on rat brain 5-HT uptake and only weakly effective on NA uptake *in vivo* (Goodlet et al., 1977). Inhibition of monoamine uptake would not therefore appear to account for the relatively high potency of mianserin as an antidepressant. In

addition, mianserin, unlike conventional tricyclic antidepressants, tends to reduce the brain concentration, but increase the turnover, of NA. This effect appears to be due to pre-synaptic $alpha_2$-antagonism (Baumann and Maitre, 1977). Such receptors are normally activ ated by released NA to inhibit further transmitter release (Starke et al., 1975; Langer, 1977). Endberg and Svensson (1980) demonstrated that systemic or microiontophoretic application of mianserin dose-dependently increased the firing rate of noradrenergic neurons in the nucleus locus coeruleus, and antagonized the inhibitory effects of the $alpha_2$-agonist clonidine, given systemically, or microiontophoretically applied NA. Blockade of pre-synaptic $alpha_2$-adrenoceptors reduces the efficiency of the physiological negative feedback system resulting in increased synaptic concentrations of the transmitter. Mianserin has also been shown to be a 5-HT antagonist in the periphery (Saxena et al., 1971) and exhibits 5-HT_{1c} and 5-HT_2 antagonist properties at central serotonin receptors (Wong et al., 1983; Fozard, 1987). In radio-ligand binding studies mianserin exhibits marked affinity for histamine H_1 receptors, $alpha_1$- and $alpha_2$-adrenoceptors and 5-HT_2 receptors (see reviews by Green and Nutt, 1983; and Leysen, 1985).

As seen with other antidepressants, chronic treatment of rats with mianserin leads to subsensitivity of the NA-stimulated adenylate cyclase system (Mishra et al., 1980).

Mianserin was chosen for examination in these studies as an example of an atypical antidepressant with an acute pharmacology that differs from that of the tricyclic antidepressants.

3.3.5 Phenelzine

The hydrazine derivative phenelzine, although rapidly metabolised (Kline and Cooper, 1980) produces irreversible, non-specific inhibition of monoamine oxidase. Thus enzyme inhibition persists after disappearance of the MAOI and its metabolites from the body. Daily treatment with MAOI thus produces cumulative effects and enzymatic activity may only be restored by resynthesis of the enzyme. Indeed Robinson et al. (1979) demonstrated that chronic treatment of rats with phenelzine, 7.5 mg kg^{-1}, resulted in maximal (i.e. at least 95%) inhibition of MAO activity after 7 days of treatment. In the same study brain tissue levels of 5-HT, NA and DA peaked within 3-7 days of treatment and then progressively decreased towards control levels even though MAO activity was still maximally inhibited, indicating adaptation by neuro-biological mechanisms to primary changes in synaptic function returning the net effect of synaptic activity to the pretreatment state. In addition, tryptophan hydroxylase activity in the mesencephalic tegmentum was, paradoxically, increased by 21 days of treatment although no consistent effect on the amount or kinetic properties of striatal or hypothalamic tyrosine hydroxylase activity was observed.

Chronic treatment with MAOI's also decreased the sensitivity of the NA-dependent adenylate cyclase system (Vetulani et al., 1976a), indicating similar chronic-treatment effects to those observed with other classes of antidepressants.

The clinical efficacy of MAOIs has been the target of some controversy. For example, the MRC trial of antidepressant treatments published in 1965 (cited by Green and Costain, 1981)

indicated that phenelzine was less effective than placebo in treating severe depressive illness. Even so, MAOI's have been widely used both in the United Kingdom and United States of America for the treatment of depression since the early 1960s, although the FDA rates phenelzine as the only MAOI effective in the treatment of depression.

Initial treatment with phenelzine, presented as the sulphate salt, starts at 15 mg (by mouth) 3 times daily and increased if necessary to 4 times daily after 2 weeks. The dose is then reduced gradually to the lowest possible maintenance dose (BNF). Paykel (1979b) suggests that the usual daily dose range is 45-75 mg.

Phenelzine was chosen for examination in these studies as an example of the non-specific irreversible MAOI's.

3.4 Doses

3.4.1 Social behaviour

All the doses of the compounds examined in these studies have been calculated on a molar basis to provide an accurate estimate of relative potency.

Clomipramine, 30 mg Kg^{-1} (equivalent to 95.3 umol Kg^{-1}) sc., depresses body posture and reflexes in the Irwin-Profile of drug activity in mice (A. Johnson, personal communication). In addition, haloperidol and diazepam are demonstrably behaviourally-depressant in rodents at 0.11 mg Kg^{-1} sc (Brown et al., 1985), equivalent to 0.29 umol Kg^{-1}, and 5 mg Kg^{-1}sc (Delini-Stula and Vassout, 1979), equivalent to 17.5 umol Kg^{-1}, respectively.

Based on this information and the respective clinical potencies of the other antidepressant compounds, the effect of acute treatment with the following doses of each drug on rodent social behaviour and exploratory locomotion were examined:

Drug	umol Kg^{-1}			(mg Kg^{-1} base)		
Clomipramine	10,	30,	90	(3.14,	9.41,	28.4)
Fluoxetine	1.1,	3.3,	10	(0.34,	1.02,	3.09)
Iprindole	1,	3,	9	(0.29,	0.86,	2.56)
Mianserin	0.33,	1,	3	(0.087,	0.26,	0.79)
Phenelzine	1,	3,	9	(0.14,	0.41,	1.24)
Haloperidol	0.11,	0.33,	1	(0.041,	0.12,	0.38)
Diazepam	3.3,	10,	30	(0.94,	2.85,	8.54)

3.4.2 Circadian locomotor activity

The daily dose of each test drug used in these studies (i.e. clomipramine, 20 mg Kg^{-1} base; fluoxetine, 2 and 6 mg Kg^{-1} pfb. and mianserin, 2 mg Kg^{-1} base) was based on previous data (Martin, 1982), on the relative potency on rodent social behaviour and, as far as possible, on clinical equivalence.

CHAPTER 4 MATERIALS

CHAPTER 4 MATERIALS

The aim of this chapter is to provide a summary of the animals, drugs and materials used throughout these studies. A detailed description of the methods employed is provided in each of the relevant chapters.

4.1 Animals

Male Wistar rats (University of Bath strain) were used in all of the studies. All animals were housed either singly or in groups according to the requirements of the particular experiment with free access to standard laboratory chow (Labsure CRM diet) and, except where indicated in the methodology, tap water.

Extreme care was taken to ensure that animals within any particular group were obtained from the same weaning colony. In the social interaction studies, however, the resident and intruder animals were weaned on the same date but obtained from different weaning colonies. These precautions ensured that as far as possible all animals used in a particular series of experiments were both age- and weight-matched and, in the social interaction studies, that the intruder rats were unknown to the resident conspecifics.

4.2 Lighting conditions

Animals used in the social interaction or exploratory locomotor activity experiments were housed in standard rodent laboratory, solid based, perspex cages (320 x 500 x 165mm) in the experimental room under reverse-daylight conditions (12h. on:12h. off; lights on 2000) for 5 weeks prior to the commencement of each experiment.

The rank order and circadian locomotor activity experiments required strict control over the lighting conditions under which the animals were maintained. Animals used in these studies were therefore housed in the environmental cabinets described in section 4.3.

Up to 4 groups of animals were used in the rank order studies at any one time. Thus while these groups were all housed under a 12h:12h light/dark cycle, the time of onset of the dark phase for each group was staggered to enable monitoring of the social behaviour during the initial 30 min. of darkness for each group in turn.

At the start of each circadian locomotor activity study the animals were housed in environmental cabinets under normal-daylight conditions (12h on:12h off; lights on 0700 or 0800). The circadian locomotor activity of the animals was then allowed to "free-run" under either complete darkness (single animals) or low intensity (2 lux) red light (grouped animals) for at least 10 days prior to the onset of chronic drug administration, and until at least 10 days following the cessation of drug treatment.

4.3 Environmental cabinets

Two types of environmental cabinet were used in these studies. Firstly, those which housed standard rodent laboratory perspex cages to control the environmental conditions of animals housed either singly for the monitoring of circadian locomotor activity (grid-based cages) or in groups for the rank order studies (solid-based cages). Secondly, that which housed the locomotor activity monitor used to record both the circadian locomotor activity of grouped animals and the exploratory locomotor activity of individual rats.

4.3.1 Circadian locomotor activity of individual rats and rank order studies

These environmental cabinets have been described by Hillier et al. (1973). They were constructed from 17mm blockboard and measured 610 x 625 x 450mm (Fig 4.1). To maximize sound and light insulation the interior of the cabinet (including the door) was covered with 12mm polystyrene sheets and 10mm commercial draft-excluder taped around the door and walls. Internal lighting (range 720-800 lux) was achieved by a 12 inch 8W white-light flourescent tube (Osram) fitted to the ceiling of the cabinet with the choke (Thorn) placed externally on a metal heat-sink to prevent excessive heating of the interior. The internal light was connected to a time switch (Sangamo, Type 5254-I-171) to provide control of the internal lighting conditions. A 30mm-bore plastic tube connected the back of the cabinet to an extractor fan (Philips, Type HR 3408) to provide adequate ventilation of the cabinet and maintenance of the interior temperature at the same level with the experimental room, i.e. 23±2 °C. Finally, a second plastic tube enabled a flexible water pipe to be connected from a reservoir placed above the cabinet to a water spout positioned in the food-hopper if required.

4.3.2 Circadian locomotor activity of grouped rats

A second environmental cabinet to house the activity monitor used to measure the circadian locomotor activity of grouped rats and the exploratory locomotor activity of individual rats, of dimensions 1300 x 900 x 1400mm, was constructed from 17mm blockboard (Fig 4.2). Maximal sound and light insulation was obtained by lining the interior of the cabinet with 12mm polystyrene sheets and the door frame with 10mm draft-excluder tape. Internal lighting (100 lux) was

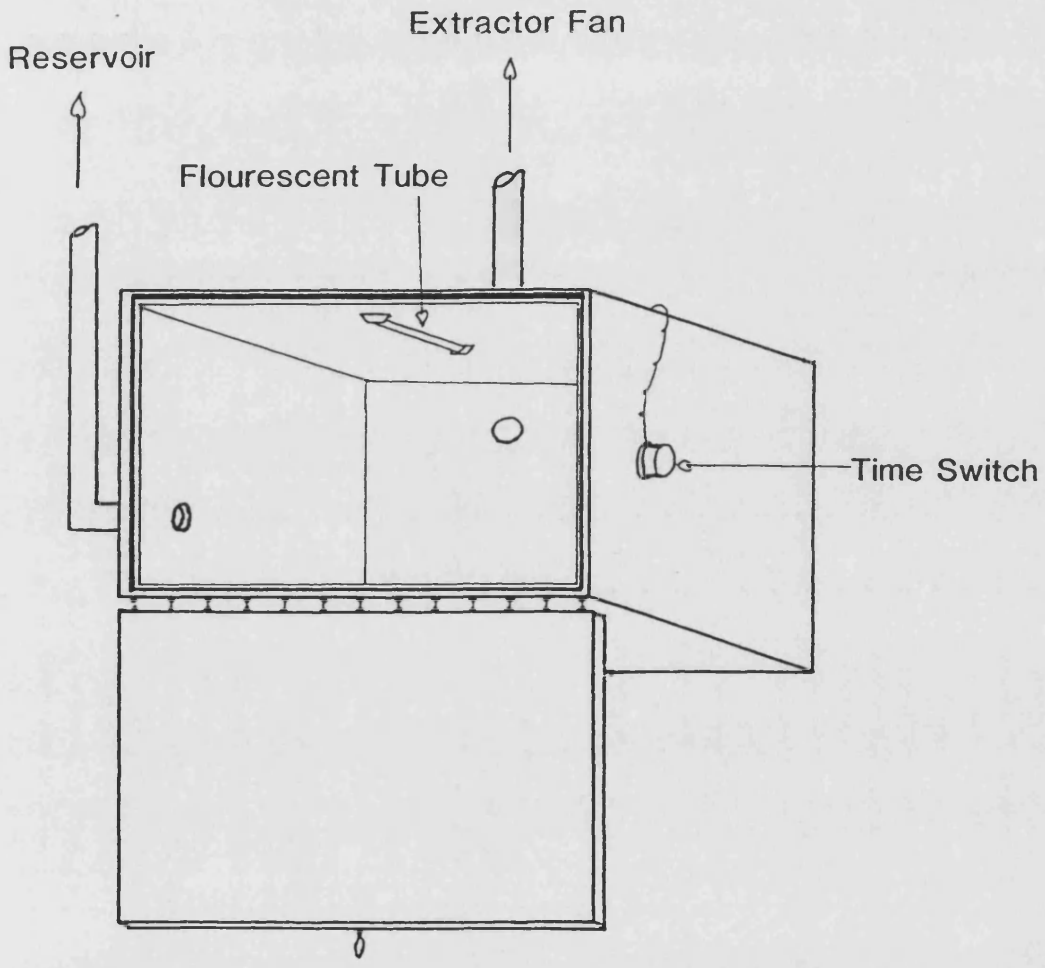


Figure 4.1 Diagram showing the main features of the environmental cabinet used in the circadian locomotor activity of individual rats and rank order studies.

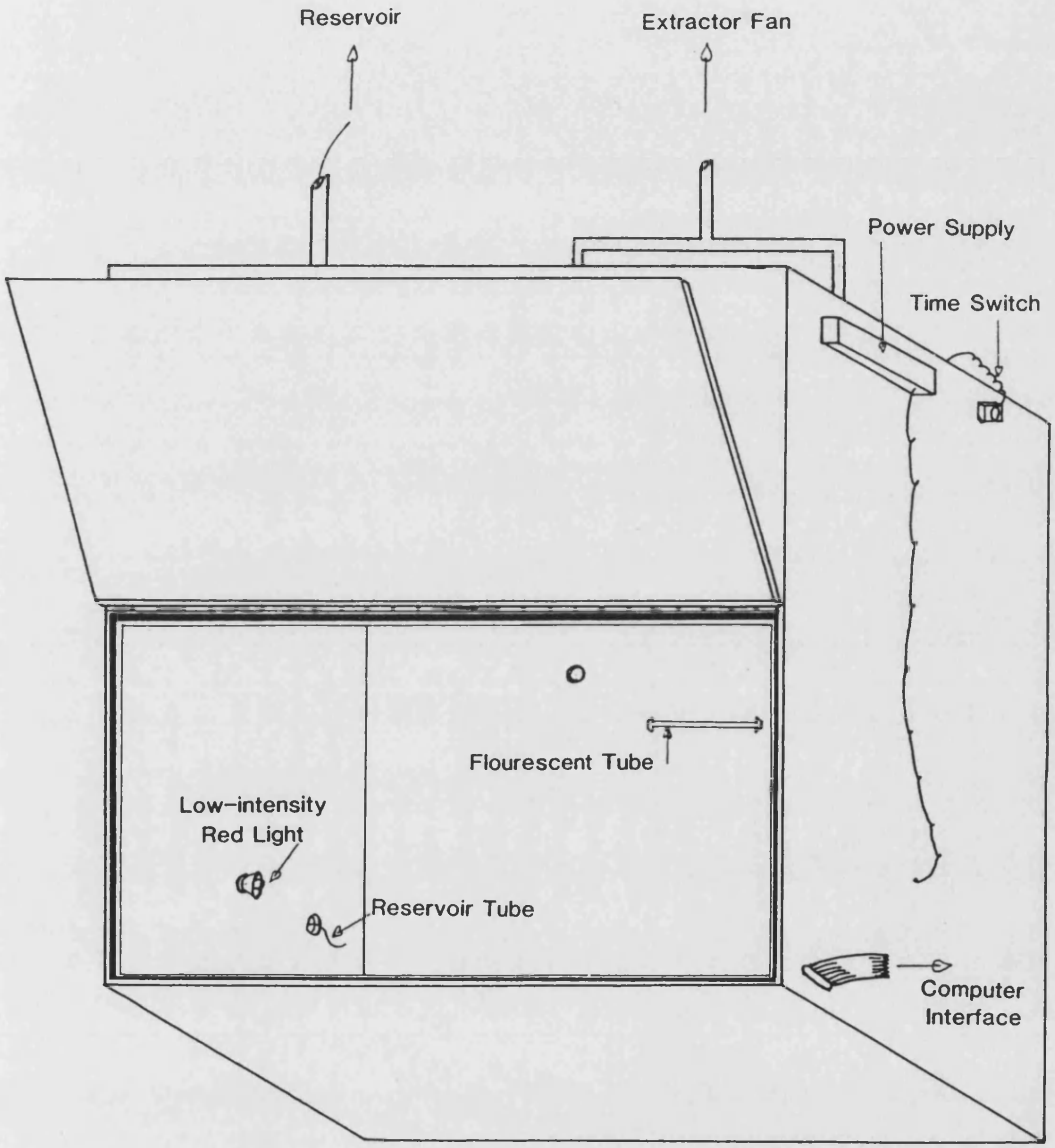


Figure 4.2 Diagram showing the main features of the environmental cabinet used in the circadian locomotor activity of grouped rats and exploratory locomotor activity studies.

achieved by a 12" 8W white-light flourescent tube (Osram) fitted to the rear wall of the cabinet with the choke (Thorn) placed externally on a metal heat-sink, to prevent excessive heating of the interior, and connected via a mains lead to a time switch (Smiths Industries, Type TS500) to provide control of the internal lighting conditions. Additional low intensity (6 lux) red lighting, to enable video recording of the animals behaviour during the dark phase of the light/dark cycle, was provided by two bayonet sockets, positioned on each side wall of the cabinet, fitted with 15W (GEC) red-light bulbs which remained on for the duration of each experiment. A 30mm-bore plastic tube connected the back of the cabinet to an extractor fan (Philips, Type HR 3408) and provided ventilation of the cabinet and maintenance of the interior temperature at the same level with the experimental room, i.e. 23±2 °C. A 120 x 70mm hole cut into the roof of the cabinet allowed the video camera to be positioned above the activity monitor to enable recording of the animals behaviour when required. The video camera was enclosed in a wooden case, constructed from 17mm blockboard, and the frame lined with 10mm draft-excluder tape to provide light and sound insulation. Finally, a second plastic tube enabled a flexible water pipe to be connected from a reservoir placed above the cabinet to a water spout positioned in the wall of the activity monitor.

4.4 Recording apparatus

4.4.1 Social behaviour

The social interaction and rank order experiments required recording of animal behaviour on to video tape, for analysis at a later date, via a video camera positioned immediately above the animals cage. Pilot recordings indicated that the normal wire cage-top, housing

the food hopper and drinking bottle, obstructed the viewing area of the camera. To overcome this problem the central section of the cage-top (305 x 365mm) was removed so that just the food hopper and the surrounding edge remained to grip the top edges of the cage. The modified cage-top was sprayed with matt-black paint to reduce reflected light into the lens of the video camera. The arrangement of the apparatus required to record rodent social behaviour is depicted in Fig 4.3.

4.4.1.1 Recording cabinet

To stop subject animals escaping by the modified cage top during recording of rodent social behaviour a cabinet was constructed from 7mm ply-wood of internal dimensions 395 x 570 x 500mm. A 200mm high opening at the base of the front panel of the cabinet allowed normal rodent cages to be introduced into the cabinet at the start of each recording session. A removable sliding partition (395 x 313mm) was then positioned 175mm from the front panel to rest on the following edge of the food hopper.

4.4.1.2 Video camera and recorder

To enable recording of rodent social behaviour during the social interaction and rank order studies a low-light/infra red video camera (ITC Ikegami CTC-4500 Type G), working-range up to 6 lux) fitted with an auto-iris lens (Koike Auto-Eye Hexanon KS, 9mm F1.4) was positioned directly above the recording cabinet. A 15W red light was positioned near the video camera and provided low red illumination (4 lux) of the recording area. The social behaviour was recorded on to VHS video tape by a JVC BR-6400TR video recorder and displayed on a video monitor (Sony PVM-201UB).

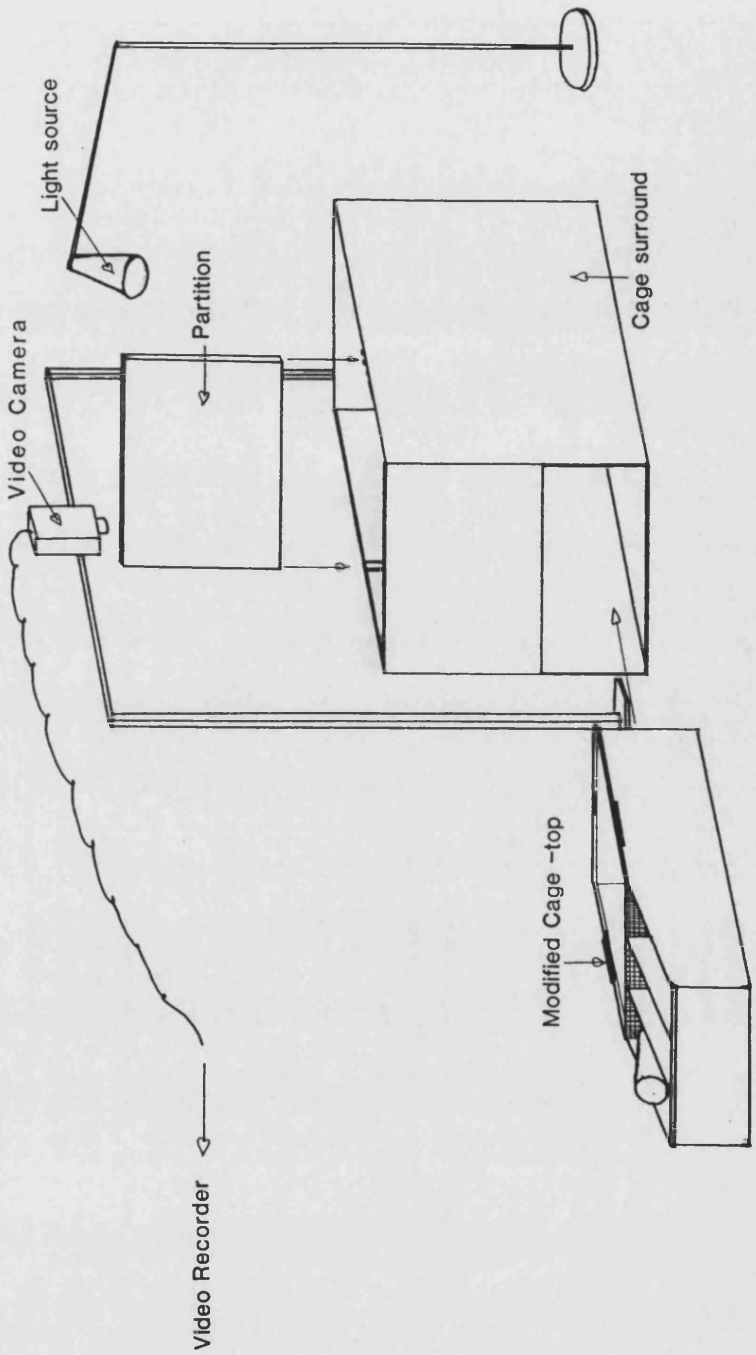


Figure 4.3 Diagram showing the arrangement of the apparatus used to record rodent social behaviour (social interaction and rank order studies).

4.4.2 Circadian and exploratory locomotor activity

4.4.2.1 Practical considerations

Documented experiments employing infrared photocell units to monitor rodent locomotor activity have typically used 1 (e.g. Costall et al., 1982) to 3 (Francis et al., 1983) photocell units (i.e. photoemitter and photodetector) positioned in the walls of an open-field chamber. Open-field monitors employing photocell units are perfectly adequate to capture the activity of isolated animals since even when the animal is at rest the monitor remains functional. When monitoring the overall activity of grouped animals, however, such monitors may be rendered ineffective if one or more of the animals rests along the path of one or more of the infrared beams. To overcome this problem a locomotor activity monitor was designed specifically to record the overall locomotor activity of grouped rats and is described in section 4.4.2.3.

4.4.2.2 Circadian locomotor activity of individual rats

The circadian locomotor activity of individual rats was measured in standard rodent laboratory grid-based perspex cages, 320 x 500 x 165mm, adapted for this purpose (Fig 4.4). One pair of infrared photo cells (i.e. one emitter and one detector) were positioned in opposing side walls of the cage 40mm above the wire-grid floor and mid-way between the food hopper and the rear of the cage (i.e. 280mm from the front of the cage). The cage was positioned in the environmental cabinet described in section 3.3.1 and the leads from the photocell pair passed through the wall of the environmental cabinet to connect with the relevent interfaces (see section 4.4.2.4). The cage was placed on wooden stilts above the sawdust-tray to enable changing of the sawdust when required without

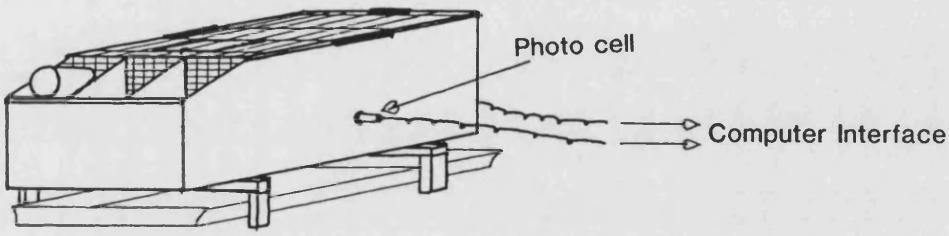


Figure 4.4 Diagram showing the locomotor activity monitor used to record the circadian locomotor activity of individual rats.

disturbing the subject animals.

4.4.2.3 Circadian locomotor activity of grouped rats and exploratory locomotor activity of individual rats

The circadian locomotor activity of grouped rats was recorded in an activity monitor (Fig 4.5), external dimensions 900 x 600 x 350mm, designed specifically to overcome the practical problems associated with the use of infrared photocells outlined above.

The walls of the monitor were constructed from 5mm black perspex and contained 7 photocell units. 3 photo-emitters and 3 photo-detectors were situated at 300mm intervals along the wall of the cage and 50mm above the wire-grid floor. The wire-grid floor was constructed from 6 stainless steel grids (290 x 290mm) which were slotted into grooves cut along the length of clear perspex batons (10 x 10mm) to create a 2 x 3 matrix. The side batons were then cemented to the side walls of the cage 50mm above the bottom edge. The cage lid was constructed from 5mm clear perspex with side strips to hold the lid tightly on the top of the cage. Integral to the lid were 2 square pillars, 120 x 120 x 300mm constructed from 5mm black perspex, each containing the 3 relevant photo-units radiating to the photo-emitter or photo-detector positioned in the wall of the cage. The 7th photocell pair, positioned in the walls of the pillars 50mm above the grid floor, were directed between the two pillars. The cables to the photo-emitters and from the photo-detectors were wired to D-sockets (Radio Spares, 15-pin) situated in the wall of the environmental cabinet. The pillars and the photocell units were so positioned as to divide the floor into 6 equal areas. A close fitting sawdust tray, constructed from 5mm black perspex, was provided which could

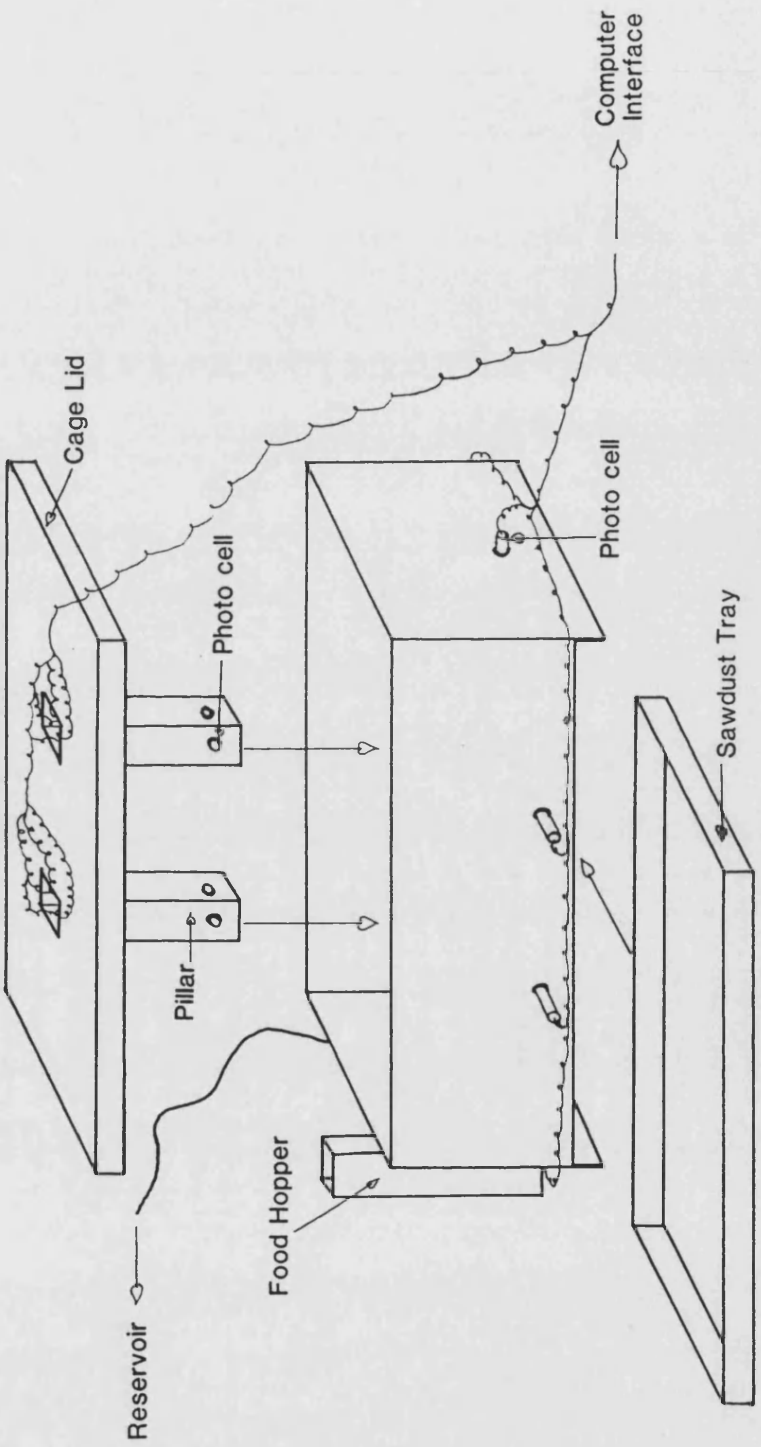


Figure 4.5 Diagram showing the locomotor activity monitor used to record the circadian locomotor activity of grouped rats and the exploratory locomotor activity of individual rats.

be removed to enable the sawdust to be changed, when required, without disturbing the subject animals. A food hopper, large enough to provide 3 animals with 7 days supply of laboratory chow (Labsure CRM diet), and drink dispenser, positioned in the wall of the cage, were provided to allow access to food and water/drug solution ad libitum. A tube from the drink dispenser was run through an air duct (50mm plastic tubing) in the wall of the environmental cabinet to allow daily measurement of fluid intake or change of drinking solution without disturbing the subject animals.

Although the primary objective in the design of this locomotor activity monitor was to overcome the practical problems outlined above, the monitor also provided a novel environment in which to monitor the exploratory locomotor activity of rats.

4.4.2.4 Infrared photocell units and computer hardware and software

The infrared photo-emitters (Scan-A-Matic; L33007) were powered by a 5 volt regulated power supply (Radio Spares switched-mode 70W). The state of each infrared photodetector (Scan-A-Matic; P33001) was continuously monitored by a BBC microcomputer (Model B) via an interface network and software designed by R. Marshall, University of Wales College of Medicine, Cardiff (Marshall et al., 1985). Any break in the infrared beam caused by an animal moving across the beam path was detected by the microcomputer, thus providing an index of locomotor activity. Accumulated counts of activity over a predetermined time period were stored on 5.25 inch floppy discs for analysis at a later date.

In addition to the software provided by Marshall a number of programs were written to enable statistical analysis of circadian locomotor activity and are described in Appendix B.

4.5 Preparation and implantation/removal of osmotic mini-pumps

Chronic administration of drugs or drug-vehicle in the social interaction, exploratory locomotor activity and rank order experiments was achieved by subcutaneous implantation of osmotic mini-pumps (Alzet) designed to deliver a constant output of drug solution over 7 (Model 2001, exploratory locomotor activity studies) or 14 (Model 2002, social interaction and rank order studies) days. In reality the pumps deliver drug solutions continuously until the pump is exhausted; up to 10 or 18 days respectively. In the social interaction and rank order studies, drugs were administered for 14 days and the behaviour of the animals examined at set time points following the cessation of drug treatment. For this reason the mini-pumps were removed 14 days after implantation.

Each mini-pump consists of a collapsible reservoir of flexible, impermeable material, surrounded by a sealed layer containing an osmotic agent; all of which is encapsulated by a semipermeable membrane. In an aqueous environment the osmotic agent imbibes water at a rate controlled by the semipermeable membrane. The hydrostatic pressure generated by the water gradually compresses the reservoir producing a constant flow of the contained drug solution through the delivery port. The 2001 and 2002 pumps deliver approximately 1 and 0.5 ul hr^{-1} <u>in vitro</u> respectively. The <u>in vivo</u> flow rate, however, is typically between 5 and 15% lower than that calculated <u>in vitro</u>. In all experiments, therefore, the <u>in vivo</u> flow rate used to determine the required drug concentration and the achieved doses

quoted in the text was assumed to be 10% lower than the *in vitro* value quoted by the manufacturers. Since the delivery rate of the contained drug solution is constant the resulting dosage is determined by the concentration of the drug loaded into the pump. Each mini-pump was prepared immediately prior to implantation. The concentration of drug loaded into each pump was calculated according to the assumed *in vivo* flow rate, the target dose to be administered over 24 hr. and the projected weight of the subject animal after 7 days of drug treatment (i.e. the mid-point of chronic drug administration in the social interaction and rank order experiments).

Prior to surgical implantation or removal of the mini-pumps each animal was anaesthetised with fentanyl/fluanisone and midazolam (see section 4.6) under low intensity red light, the current lighting conditions for that group of animals. Once anaesthetised the animals were removed to the operating suite.

4.5.1 Implantation

After shaving and cleansing the surgical site with hibitane a small skin incision was made adjacent to the site chosen for pump placement (invariably along the mid-line of the back). A haemostat was inserted in one end of the wound to create a subcutaneous pocket for the pump. The pump was then inserted with the delivery portal away from the wound site, following which the site was dusted with cicatrin antibiotic powder and the wound sealed with wound clips (Mikron; 9mm Auto-clips).

4.5.2 Removal

The area of the skin near the portal end of the mini-pump was shaved

and cleansed with hibitane. A small incision was made and the mini-pump removed through the wound. Following removal the placement site was inspected for tissue invagination, necrosis or infection. The wound was then dusted with cicatrin and sealed with wound clips. It should be noted at this point that no tissue damage resulting from implantation of the mini-pumps or chronic drug delivery by this method was observed.

After each operation the animals were replaced in their home cage, returned to the respective experimental room and placed on a heated bed to aid recovery from the anaesthesia.

4.6 Drugs and chemicals

Table 4.1 summarizes the drugs and chemicals used during these studies together with their source and, where applicable, the vehicle in which they were prepared.

The choice of each drug-vehicle was made considering that in the social interaction, exploratory locomotor activity and rank order studies each compound was to be administered chronically via subcutaneously implanted osmotic mini-pumps which required comparatively high drug concentrations. Those compounds available as the hydrochloride (i.e. clomipramine, iprindole and mianserin) or sulphate (i.e. phenelzine) salts were adequately soluble in H_2O. Haloperidol and fluoxetine, available as the pure free base (pfb), were sufficiently soluble in 0.1M tartaric acid. Diazepam (pfb), which was found to be insoluble in H_2O or 0.1M tartaric acid at the concentrations required for chronic administration via osmotic mini-pumps, was solubilized in 40% v/v propylene glycol,

10% v/v ethanol, 1.5% v/v benzyl alcohol and made up to volume (i.e. 48.5% v/v) with 5% w/v sodium benzoate in H_2O according to the vehicle described by Turmel and De Montigny (1984). Where appropriate this vehicle (denoted diazepam-vehicle in the text) was employed as the control vehicle for the experiments examining the effects of diazepam upon social behaviour or exploratory locomotor activity.

All drug concentrations in the text are expressed in terms of the free base.

In the social interaction, exploratory locomotor activity and rank order studies all test drugs or vehicle were administered subcutaneously either acutely, dose volume 1 ml Kg^{-1}, or chronically via osmotic mini-pumps as described above.

Prior to implantation or removal of the osmotic mini-pumps all rats were anaesthetised with fentanyl/fluanisone and midazolam prepared as follows; 4 ml of the commercially available fentanyl citrate, 0.315 mg ml^{-1}, and fluanisone (pfb), 10 mg ml^{-1}, mixture (Hypnorm) was diluted with 8 ml of sterile H_2O, following which 4 ml of midazolam hydrochloride (Hypnovel), 5 mg ml^{-1} (base), was added. The final anaesthetic cocktail thus contained fentanyl citrate, 0.079 mg ml^{-1}, fluanisone, 2.5 mg ml^{-1}, and midazolam, 1.25 mg ml^{-1}. The anaesthetic cocktail was administered intraperitoneally at a dose volume of 0.275 ml per 100g body weight. This dose was determined following strain-testing for induction of anaesthesia in the Bath University strain of Wistar rats and provided at least 30 min. of surgical anaesthesia.

In the circadian locomotor activity experiments all drugs used were presented to the animals in the drinking water. Clomipramine and mianserin were dissolved in "polished" H_2O (see below for definition) while fluoxetine was initially dissolved in a minimal quantity of 0.1M tartaric acid and then made up to volume with "polished" H_2O.

Polished H_2O was obtained from the Milli-Q Reagent water system (Millipore Corp.)

Sterile H_2O was obtained by sterilising double-distilled water in an autoclave.

Drug/Chemical	Source	Vehicle
Clomipramine hydrochloride	Geigy *	H_2O (polished)
Diazepam (pfb)	Roche *	40% propylene glycol 10% ethanol 1.5% benzyl alcohol 48.5% sodium benzoate (5% in H_2O, polished)
Fluoxetine (pfb) (LY 110140)	Eli-Lily *	a) 0.1M tartaric acid b) Minimal 0.1M tartaric acid + H_2O (polished) to quantity
Haloperidol (pfb)	Searle *	0.1M tartaric acid
Iprindole hydrochloride	Wyeth *	H_2O (polished)
Mianserin hydrochloride	Beecham *	H_2O (polished)
Phenelzine sulphate	Sigma	H_2O (polished)
Cicatrin	Calmic	
Fentanyl citrate/ Fluanisone (pfb) (Hypnorm)	Janssen	H_2O (sterile)
Midazolam hydrochloride (Hypnovel)	Roche	H_2O (sterile)
Benzyl alcohol	Aldrich	
Ethanol 95%	BDH	
Propylene glycol (pfs)	BDH	
Sodium benzoate (pfs)	BDH	
Tartaric acid (pfs)	BDH	H_2O (polished)

Table 4.1 Drugs and chemicals

* Gifts from source which are gratefully acknowledged

a) Social interaction, exploratory locomotor activity and rank order studies

b) Circadian locomotor activity studies

CHAPTER 5 SOCIAL INTERACTION STUDIES IN THE RAT

CHAPTER 5 SOCIAL INTERACTION STUDIES IN THE RAT

5.1 Introduction

Grant and Mackintosh (1963) described the elements of social behaviour in the laboratory rat, mouse, hamster and guinea-pig, in terms of postures and acts, most of which were common to all four species. Grant (1963) demonstrated that the consecutive behavioural elements of the rat do not occur at random and may therefore have related but not identical functions. Such actions were shown to fall into behavioural pathways indicating an inherent progression from one behaviour to the next. Fig. 5.1 summarizes the individual behavioural postures and how they may be grouped into behavioural pathways according to the motivational category in which they occur. Silverman (1965) demonstrated that the ethological approach of Grant and Mackintosh could be employed to study the effect of drugs on rodent social behaviour. Such an approach to the study of drug activity on rodent behaviour is attractive since it does not resort to the modification of behaviours induced by a second drug. Thus the effects of a drug per se. on rodent behaviour may be studied.

5.1.1 Behavioural postures of rodent social behaviour

The following list describes the rodent social behavioural postures modified from Grant and Mackintosh (1963) grouped according to the respective motivational categories as employed by Silverman (1965). The majority of definitions of the behavioural postures used in these studies are as described by Silverman, however for practical reasons a few modifications have been made.

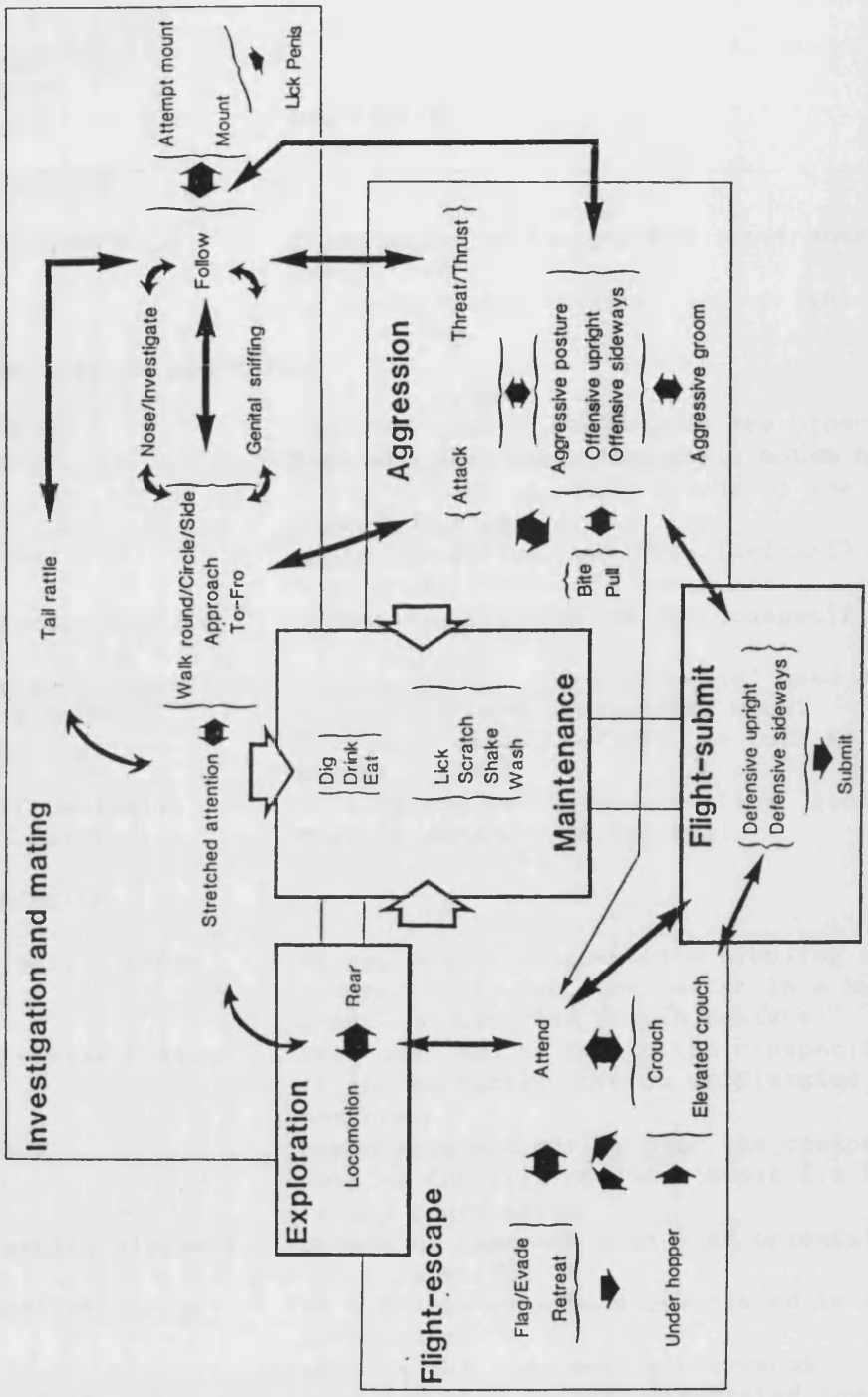


Figure 5.1 Simplified schematic representation of the pathways of social behaviour of the rat.

Individual behaviours or postures are grouped according to motivational category. Closed arrows indicate the normal progression of behaviours. Open arrows indicate displacement activity.

Adapted from Grant (1963).

MOTIVATIONAL CATEGORY Element	Description
4 FLIGHT-SUBMIT	
Defensive Sideways	Broadside stance with the ventral surface rotated to the conspecific but with the head orientated away from the other rat
Defensive Upright	On hindlegs facing the conspecific (as Offensive Upright) but the head is orientated away from the conspecific
Submit	Lies flat on back with ventral surface towards the conspecific
5 FLIGHT-ESCAPE	
Attend	Orientation directed towards the other rat, often with Crouch
Crouch	On all four paws with shoulders lowered, sometimes with one forepaw raised. The hind legs may be extended
Elevated Crouch	As Crouch but on hind legs with forepaws on the wall of the cage. May be envisaged as Crouch with Rear
Flag and Evade	Head and forebody turned away from the conspecific, sometimes followed by rapid movement away (Retreat)
Retreat	Rapid movement away from the conspecific usually to a place of safety
Under food hopper	Place to retreat to
6 MAINTENANCE	
Digging	Burrow into the sawdust on the floor of the cage
Drinking	Self explanatory
Eating	Self explanatory
Licking	Lick's own body fur
Lick Penis	Invariably follows Attempt Mount or Mount
Scratching	Scratch own body fur usually with hind limbs
Shaking	Wriggles body
Washing	Wipe face with licked forepaws

The recording environment used in these studies consisted of an open topped cage, to enable unrestricted viewing of the experimental area, surrounded by a ply-wood enclosure (see section 4.4.1 for a complete description). This allowed the animals to explore not only the cage floor but also around the lip of the cage wall. In addition, full rearing behaviour could be displayed from both the

cage floor and the cage wall. Animals could also display a Crouch-like posture while standing against the cage wall. The term "Crouch" in these studies was therefore taken to include Silverman's definition of both Crouch and Elevated Crouch (where the legs are extended), whereas the term "Elevated Crouch" was employed to describe the static defensive posture against the side of the cage. Lastly, those postures defined as 'residual' by Silverman were not recorded since they were observed very infrequently, if at all, and when they were observed they appeared to occur more by chance than due to any motivational drive by either animal. Vocalization was not recorded.

5.2 Methods

5.2.1 Experimental Design

5.2.1.1 Methodological considerations

The purpose of these studies was to observe the social behaviour of rats in a test situation that optimised the level of physical contact between the subjects. The housing conditions, familiarity of the partner and lighting levels have all been shown to affect social interaction (File and Pope, 1974). Rats housed singly demonstrate higher levels of both active and passive contact with partner animals than those housed in pairs. Unfamiliar partners increased the level of active, but not passive, contact as compared to when the partner was familiar. Latane (1969) suggests that rats require physical contact and that requirement is increased when rats are housed in isolation. Increasing the level of illumination of the test box results in a decrease in the time spent in social interaction (File and Hyde, 1978). In addition Latane (1969) reported low levels of aggression when two rats were placed in an open field which File and

Pope (1974) suggest is due to the lack of territorial advantage between the animals. Only when one rat intrudes on the other's territory does social interaction include measureable levels of aggressive encounters.

File and Pope (1974) suggest that the effect of a centrally acting compound on social behaviour may be affected by the social rank of the treated animal. Housing the rats in groups of 3 to 6 animals per group allows a stable rank order to be established (Grant and Chance, 1958). Silverman (1965) argues that the presence of a stable rank order is useful, and this is seen to be especially so where repeat-testing occurs, since only then are the various rank positions spread equally throughout all treatment groups.

Considering these observations and the need to provide an experimental environment to optimise the level of social interaction, all experiments were designed under the following conditions:

1 All animals were housed in closed social groups prior to and during each experiment in order to allow a stable social structure to develop and be maintained during the experimental period.
2 Test animals (i.e. resident) were isolated before each experimental day, while intruder animals were housed in social groups.
3 In each social interaction test the animals were unfamiliar to each other.
4 All experiments were carried out under low illumination.
5 All experiments were carried out in familiar conditions (i.e. in laboratory cages).

6 All experiments were performed during the dark phase of the light-dark cycle when rats are normally most active.

The ontogeny of rodent behaviour is dependent on social interaction experienced prior to and following weaning (Bolles and Woods, 1964; Einon et al., 1978), thus animals within any resident or intruder group were obtained from the same group of weaners. In order to maintain constancy of social experience each experiment commenced at the same time point following weaning.

All social interaction studies were carried out with the resident animals' home cage positioned inside the recording cabinet described in section 4.4.1

Preliminary observations (data not shown) indicated that isolated animals resident in their home cage for 3 days prior to observation acclimatized to the recording cabinet within 20 minutes following positioning of the home cage in the cabinet. During the first 10 minutes resident animals showed periods of exploration (locomotion and rearing) interspersed with progressively longer periods of grooming and rest under the food hopper. By 30 minutes following introduction resident animals were invariably quiescent. Conversely, animals previously housed in groups exhibited high levels of activity for at least 20 minutes following introduction to a new cage positioned in the recording cabinet. Such activity consisted of almost continuous exploratory behaviour interspersed with short periods of grooming behaviour, which in this situation may be indicative of displacement activity implying motivational conflict (Bastock et al., 1953; Silverman, 1965).

Considering these observations social interaction experiments were designed as described below.

Initially a study of intruder-alone behaviours was performed in order to quantify the baseline behaviour of intruder animals without interference from the resident animal (see section 5.2.2.1).

The series of acute drug studies were designed to identify minimum effective doses on the resident animals' behavioural profile in the social interaction test (section 5.2.2.2). These doses were then administered on a daily basis, via osmotic mini-pumps (see sections 3.1 and 4.5) to study the effect of chronic treatment on the behavioural profile resident rats (section 5.2.2.3).

In order to study the progression of any behavioural change induced by chronic drug treatment a repeat-testing regime was employed in the chronic studies. To maintain consistency between chronic and acute drug studies a similar repeat-testing regime was used in all acute drug studies. It should be borne in mind that previous experience of social behaviour may condition subsequent behaviour in an agonistic situation (Taylor, 1979; Bolhuis et al., 1984), however, since drug studies employed animals maintained in closed social groups the effects of conditioning would be spread equally thoughout each treatment group.

Intruder animals did not receive test-drugs or drug-vehicle in either series of experiments.

5.2.1.2 Subjects

Two groups of 8 rats per group (designated "resident" and "intruder" respectively) (age-matched male Wister, Bath University stock, 100-150g when removed from the colony), obtained from different parental groups to ensure that resident animals had never been in contact with the animals in the intruder group, were maintained under reverse-daylight conditions (12h. on/12h. off, lights on 2000h) for at least 5 weeks before the start of the experimental period with standard laboratory chow (Labsure CRM diet) and water available ad libitum. Each group of 8 rats were further subdivided into 2 groups of 4 animals at least three weeks prior to the experiment.

5.2.2 Basic Methodology

With one exception (see section 5.2.2.3) each group of animals in the social interaction studies was tested on 4 occasions only with all social interaction experiments performed on a strict weekly cycle. During each week before testing all animals were handled individually on at least 3 occasions in order to habituate each animal to the experimenter. The resident rats were separated 3 days before each test day and housed individually with food and water available ad libitum.

In order to enable identification all resident animals were head-marked immediately prior to each experiment. At the start of each social interaction test the resident animal in its home cage was positioned inside the recording cabinet for 30 minutes prior to introduction of the relevant intruder animal to allow adequate acclimatization of the resident rat to the recording cabinet. At the

end of the acclimatization period the intruder rat was introduced into the resident animals home cage and the resulting social interaction recorded on video tape for the following 10 minute period (see section 5.2.2.3).

At the end of each recording all animals were returned to their respective group cages in order to maintain the social structure of each group.

5.2.2.1 Analysis Of Intruder-Alone Behaviours

Immediately prior to introduction of the intruder animal to the resident animals' cage the resident rat was removed and returned to its social group. The intruder animals behaviour was then recorded on to video tape for the following 10 minute period. Each intruder animal was monitored on one occasion only.

5.2.2.2 Effect of acute drug treatment of the resident rat

Resident animals were dosed subcutaneously (sc) with drug or vehicle (dose volume of 1ml Kg^{-1}) and returned to their individual home cage. All drugs and doses used in the acute studies were chosen according to their known clinical use and pharmacological effects (see Table 5.1 and sections 3.3 and 3.4), and were administered on a mol Kg^{-1} body weight basis in order to obtain an accurate indication of potency.

All acute drug studies were performed according to the experimental matrix indicated in Table 5.2, with two groups of resident rats being tested concurrently. The experimental matrix ensured that each animal received the drug vehicle and 3 doses of drug, and came into

contact with each of the corresponding intruder animals. Likewise each intruder animal came into contact with each drug treatment. Such a balanced dosing schedule also ensured the effect of rank position in both the resident and intruder groups was spread equally throughout all dosing groups.

5.2.2.3 Effect of chronic drug or drug-vehicle treatment of the resident rat

In these experiments resident animals were tested on the first occasion without any drug treatment, following which Alzet mini-osmotic pumps containing drug or vehicle were implanted sc under fentanyl/fluanisone/midazolam aneasthesia (see sections 4.5 and 4.6) and then returned to their group cage. Social interaction tests were carried out after 7 and 14 days of drug treatment and 7 days following removal of the mini-pumps. The mini-pumps were removed under fentanyl/fluanisone/midazolam anaesthesia immediately following the social interaction test on day 14 of drug treatment. Control experiments, where the mini-osmotic pumps contained the respective drug-vehicle, were designed as follows: experiments to study the effect of chronic administration of clomipramine, iprindole, mianserin or phenelzine were controlled by using pumps containing polished H_2O; experiments studying the effect of chronic administration of fluoxetine or haloperidol were controlled by using pumps containing 0.1M tartaric acid; while the effect of chronic administration of diazepam was controlled by using pumps containing diazepam-vehicle as described in section 4.6.

All chronic drug studies were carried-out according to the experimental matrix indicated in Table 5.3, with two groups of

resident rats being tested concurrently. As in the acute studies the experimental matrix ensured that each resident animal came into contact with each of the corresponding intruder animals.

The data obtained at 7 days following the cessation of chronic phenelzine treatment (see section 5.5.3.5) indicated that the behavioural profile of the resident rats had not returned to that observed prior to drug treatment. A further social interaction study was therefore performed at 14 days post-phenelzine treatment where the intruder animals were obtained from the other, concurrent, age-matched intruder group.

Drug (Doses umol/kg)	Vehicle	Clinical use	Acute mode of action
Clomipramine (10, 30, 90)	H2O	Antidepressant	Inhibition of 5-HT and NA reuptake
Fluoxetine (1.1, 3.3, 10)	Tartaric acid	Potential antidepressant	Inhibition of 5-HT reuptake
Iprindole (1, 3, 9)	H2O	Antidepressant	Not Known
Mianserin (0.33, 1, 3)	H2O	Antidepressant	Antagonist at $5\text{-}HT_{1c}$, $5\text{-}HT_2$ and alpha-NA receptors
Phenelzine (1, 3, 9)	H2O	Antidepressant	Non-specific inhibitor of monoamine oxidase
Diazepam (3.3, 10, 30)	see section 4.6	Anxiolytic	Facilitation of GABA transmission
Haloperidol (0.11, 0.33, 1)	Tartaric acid	Antipsychotic	Antagonist at D2-DA receptors

Table 5.1 Summary of drugs studied in the social interaction test

		Resident Animal No.			
		1	2	3	4
Week 1	Treatment	A	B	D	C
	Intruder No.	1	2	3	4
Week 2	Treatment	C	D	B	A
	Intruder No.	2	1	4	3
Week 3	Treatment	B	A	C	D
	Intruder No.	3	4	1	2
Week 4	Treatment	D	C	A	B
	Intruder No.	4	3	2	1

Table 5.2 Acute Experimental Matrix

		Resident Animal No.			
		1	2	3	4
Week 1	Pretreatment				
	Intruder No.	1	2	3	4
Week 2	7 days treatment				
	Intruder No.	2	1	4	3
Week 3	14 days treatment				
	Intruder No.	3	4	1	2
Week 4	7 days post-treatment				
	Intruder No.	4	3	2	1

Table 5.3 Chronic Experimental Matrix

5.3 Identification and capture of rodent social behaviour

5.3.1 Video analysis

The observation and recording of the various elements of rodent social activity indicated in section 5.1.1 are necessarily both intense and time consuming. The various elements may change very quickly since the behaviour of each animal is stimulated by, and in part dependent on, the behaviour exhibited by the conspecific. It is important to analyse the behaviour of both animals since drug-induced changes in the resident animals' behavioural profile produces concomitant changes in the behavioural profile of the conspecific intruder (Silverman, 1965). Recording each social interaction epoch on to video tape allows for analysis at a later date, and for the recording to be replayed in order to analyse the behaviour of each animal in turn. Thus an infrared video camera was positioned immediately above the recording cabinet and the recording area illuminated in low red light by means of a 15W red bulb suspended above the camera. The level of illumination was measured at 4 lux, which was sufficient for the camera to obtain adequate recordings of rodent social behaviour without interfering with the reverse-daylight cycle in which the experimental rats were maintained (see also section 4.4.1).

5.3.2 Capture of behavioural data

To ease the handling of data arising from these studies a computer programme was developed for use with the BBC microcomputer (see Appendix A for details).

The programme, entitled Capture of Rodent Interaction Behaviours (C.R.I.B.), allows each of the behavioural postures of firstly the

resident and then the intruder animal to be identified and recorded as they are observed on the recording, together with the latency of each behaviour.

The resulting data was then stored on disk for automated collation and statistical analysis at a later date.

5.4 Statistical Analysis

5.4.1 Comparison of behavioural profiles exhibited by resident and intruder rats during social interaction

The data for each motivational category were summed and the resulting behavioural profiles compared by X^2 analysis.

5.4.2 Effect of acute or chronic drug treatment on the behavioural profile exhibited by resident and non-drugged intruder rats during social interaction

The data for each social interaction were collated and the number of behaviours within each motivational category expressed as a percentage of the respective total number of behaviours observed. The mean and standard error of the mean (sem) for the percentage values of each motivational category within each treatment group were calculated. Data for each motivational category were expressed as the percentage of total behaviours observed since variation in the total number of behaviours may occur without any change in the proportion of each motivational category.

A number of different statistical methods have been applied to social interaction data including X^2 analysis, t-test and the Mann-Whitney U-test (Silverman, 1978). Data arising from these studies were not

subjected to parametric statistical analysis because of the sampling technique employed to obtain the data. In these experiments any significant differences between treatments were determined by use of the non-parametric Mann-Whitney U-test, although it should be noted that the use of X^2 analysis would be equally relevent.

Where applicable the dose of a particular drug to reduce a behaviour by 50% (i.e. ID_{50}) was calculated by use of the least-squares method. Where such values are quoted in the text the 95% fiducial limits are also indicated in parenthesis.

5.5. Results

5.5.1 Preliminary behavioural studies

5.5.1.1 Behavioural profiles of resident and intruder rats exhibited during social interaction

Fig. 5.2 summarizes the control behavioural profile of both resident and intruder animals, where the resident animal was treated with H_2O, 1ml Kg^{-1} sc., 30 mins prior to introduction of the intruder animal. X^2 analysis indicates the behavioural profiles of the resident and intruder to be highly significantly different (X^2=5886, df=5, $p<0.001$).

5.5.1.1.1 Resident animals

The majority of the resident animals behaviours observed during the monitoring period were orientated towards investigation of the intruder animal (52.7±2.1% of total behaviours) or it's environment (19.5±1.1%). Investigation of the intruder occasionally led to aggressive acts against that animal (17.4±2.1%), while the remainder of the motivational categories occurred with low intensities (flight-escape, 4.5±0.3%; maintenance, 3.5±0.4%; flight-submit, 2.5±0.4%).

5.5.1.1.2 Intruder animals

Intruder animals predominantly exhibited flight behaviour (42%) in response to its investigation by the resident animal. Flight behaviour may be subdivided depending whether the interaction of the animals results in their spatial seperation (i.e. flight-escape, 30.5±1.0%) or remaining in close contact, whereupon the intruder animal succumbs to the resident animals activity (i.e. flight-submit, 11.5±0.5%). Intruder animals exhibited high levels of exploratory

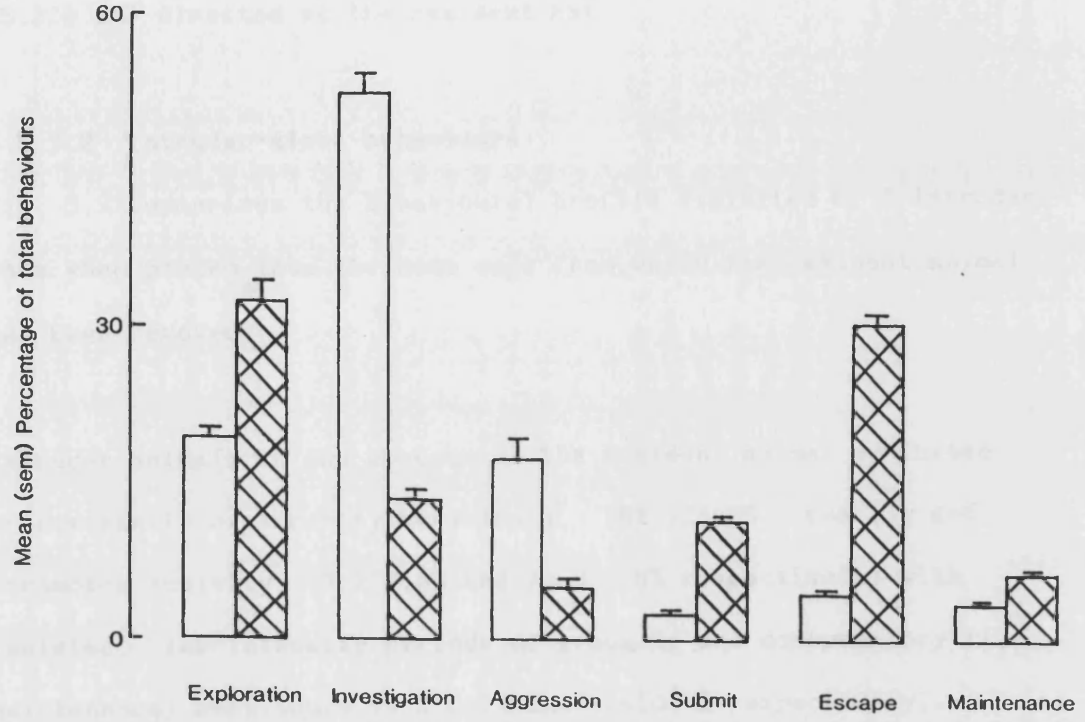


Figure 5.2 Behavioural profile of resident and intruder rats during social interaction.

Values indicate mean (±sem) percentage of total behaviours for each motivational category, where N=4 experiments (8 animals per experiment).

Open columns; resident rats. Hatched columns; intruder rats.

X^2=5886, df=5, $p<0.001$

Total number of behaviours: Resident rats, 2993±372; Intruder rats, 2608±329

activity towards the new environment (32.6±1.7%), together with lower levels of investigatory behaviour directed at the resident animal (13.3±1.3%). Intruder animals demonstrated only low levels of maintenance behaviour (6.3±0.4%) and aggressive behaviour (5.2±0.8%) directed at the resident rat.

5.5.1.2 Intruder-alone behaviours

Fig. 5.3 summarizes the behavioural profile exhibited by 7 intruder rats when placed into the home cage from which the resident animal had been removed.

Intruder animals in the absence of the resident animal exhibited predominantly exploratory behaviours, (87.7±2.2%; rearing and locomotor activity, 57.2±2.8% and 30.5±1.6% respectively) with isolated, low-intensity periods of grooming and consummatory (i.e. maintenance) behaviours (9.3±0.7% and 2.9±0.4% respectively).

5.5.2 Effect of acute drug treatment on the social interaction behavioural profiles of resident and non-drugged intruder rats

5.5.2.1 Clomipramine

The behavioural profiles exhibited by resident rats following 30 minutes subcutaneous pre-treatment with H_2O, 1ml Kg^{-1} or clomipramine, 10-90 umol $Kg^{-1}$and by non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.4 and Table 5.4 respectively.

5.5.2.1.1 Resident animals

Clomipramine, 10-90 umol Kg^{-1} sc., induced a dose-related reduction in the level of aggression (ID_{50} = 29.5 (14.3,60.0) umol Kg^{-1})

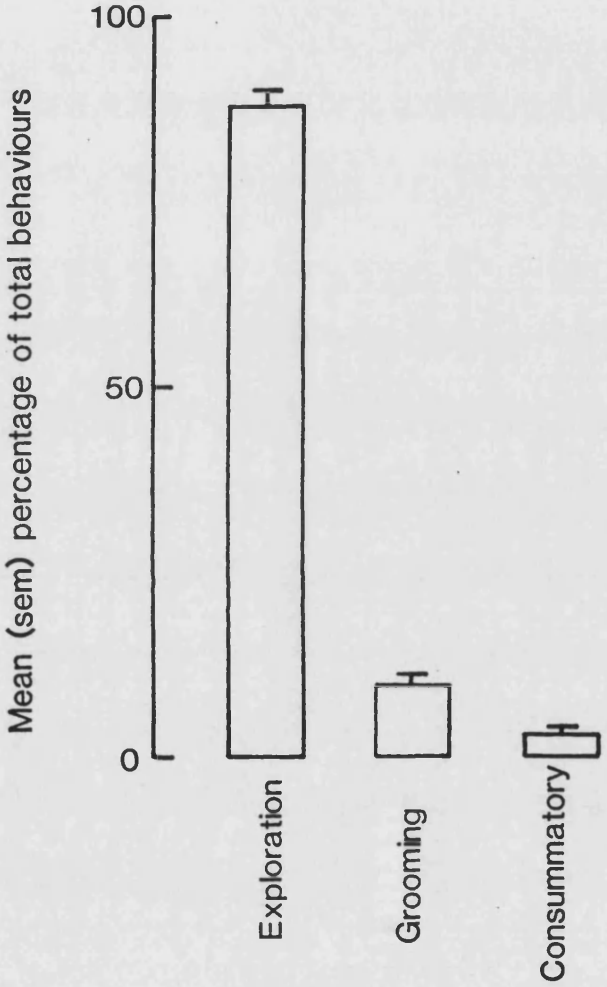


Figure 5.3 Intruder-alone behaviours.

Columns indicate mean (±sem) percentage of total number of behaviours for each motivational category.

N=7 animals

Total number of behaviours; 237±14

directed towards the conspecific intruder, concomitant with dose-related increases in both flight-submit and flight-escape behaviours. Clomipramine, 90, but not 10 or 30, umol Kg^{-1}, significantly increased the level of environmental exploration. Clomipramine, 10-90 umol Kg^{-1}, had no effect on the level of investigatory behaviour directed at the intruder rat nor on the level of maintenance behaviour.

Only clomipramine, 90 umol Kg^{-1}, significantly reduced the total number of behaviours exhibited by the resident rat.

5.5.2.1.2 Intruder animals

The levels of both flight-submit and flight-escape behaviours exhibited by intruder rats progressively decreased, while that of exploratory behaviour progressively increased, with increasing doses of clomipramine, 10-90 umol Kg^{-1}, administered to the resident rat. In addition, the level of investigatory behaviour directed at the resident rat increased (although not significantly) following treatment of the resident rat with clomipramine, 30 and 90 umol Kg^{-1}. The levels of aggressive and maintenance behaviours and the total number of behaviours exhibited by intruder rats showed no consistent change in response to the clomipramine-induced change in the behavioural profile of resident rats.

Figure 5.4 Effect of acute treatment with clomipramine on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats.

Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

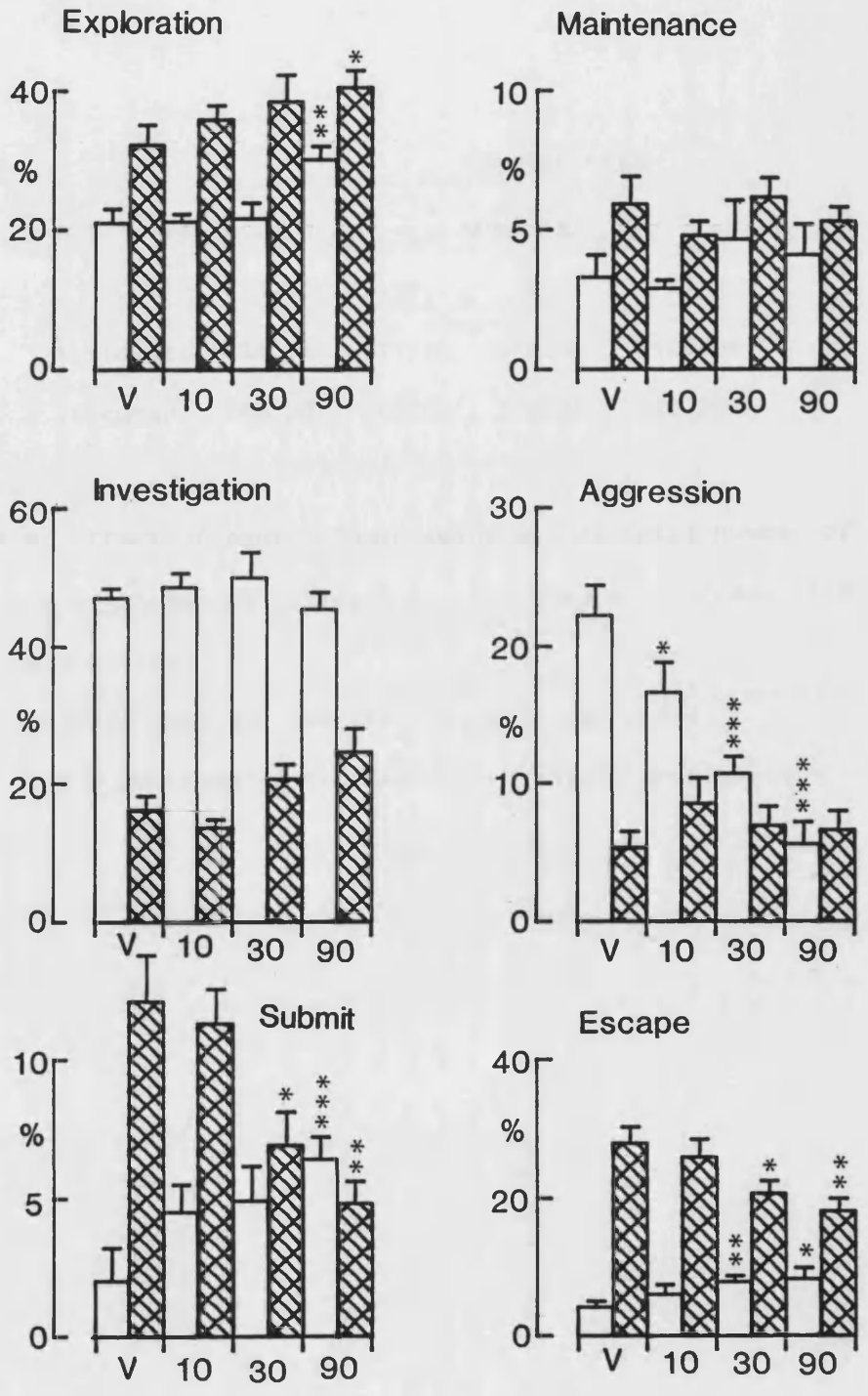
N=8 animals per group.

V, drug-vehicle (H_2O, 1 ml Kg^{-1} sc).

10, 30, 90, indicate clomipramine doses (umol Kg^{-1} sc).

Pre-treatment time=30 min.

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to respective vehicle treatment.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	Clomipramine umol Kg^{-1} sc.			
	H_2O	10	30	90
Resident	434±21	417±16	376±30	340±26a
Intruder	348±29	365±33	378±24	360±26

Table 5.4 Effect of acute clomipramine on the total number of behaviours exhibited by resident and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

MWUT : a, $p<0.05$ compared to respective vehicle treatment.

5.5.2.2 Fluoxetine

The behavioural profiles exhibited by resident rats following 30 minutes subcutaneous pre-treatment with 0.1M tartaric acid, 1ml Kg^{-1}, or fluoxetine, 1.1-10 umol Kg^{-1}, and by non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.5 and Table 5.5 respectively.

5.5.2.2.1 Resident animals

Fluoxetine, 1.1 and 3.3 umol Kg^{-1} sc, induced a dose-related reduction in the level of aggression (ID_{50} = 3 umol Kg^{-1} approximately) directed towards the conspecific intruder concomitant with a dose-related increase in the level of flight-escape behaviour. Fluoxetine, 10 umol Kg^{-1} sc also significantly reduced the level of aggressive behaviour and significantly increased the level of flight-escape behaviour, but only to those levels observed following fluoxetine, 3.3 umol Kg^{-1} sc. Fluoxetine, 1.1-10 umol Kg^{-1} sc, induced a dose-related increase in the level of flight-submit behaviour which was only significant following treatment with the highest dose tested. Fluoxetine, 10, but not 1.1 or 3.3 umol Kg^{-1} sc, significantly reduced the level of investigation directed at the conspecific intruder. No significant effect of fluoxetine treatment was observed on environmental exploratory behaviour, maintenance behaviour nor the total number of behaviours observed at any of the doses tested.

5.5.2.2.2 Intruder animals

No consistent or significant change was observed in the behavioural profile nor in the total number of behaviours exhibited by non-drugged intruder rats during social interaction with resident

Figure 5.5 Effect of acute treatment with fluoxetine on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats.

Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

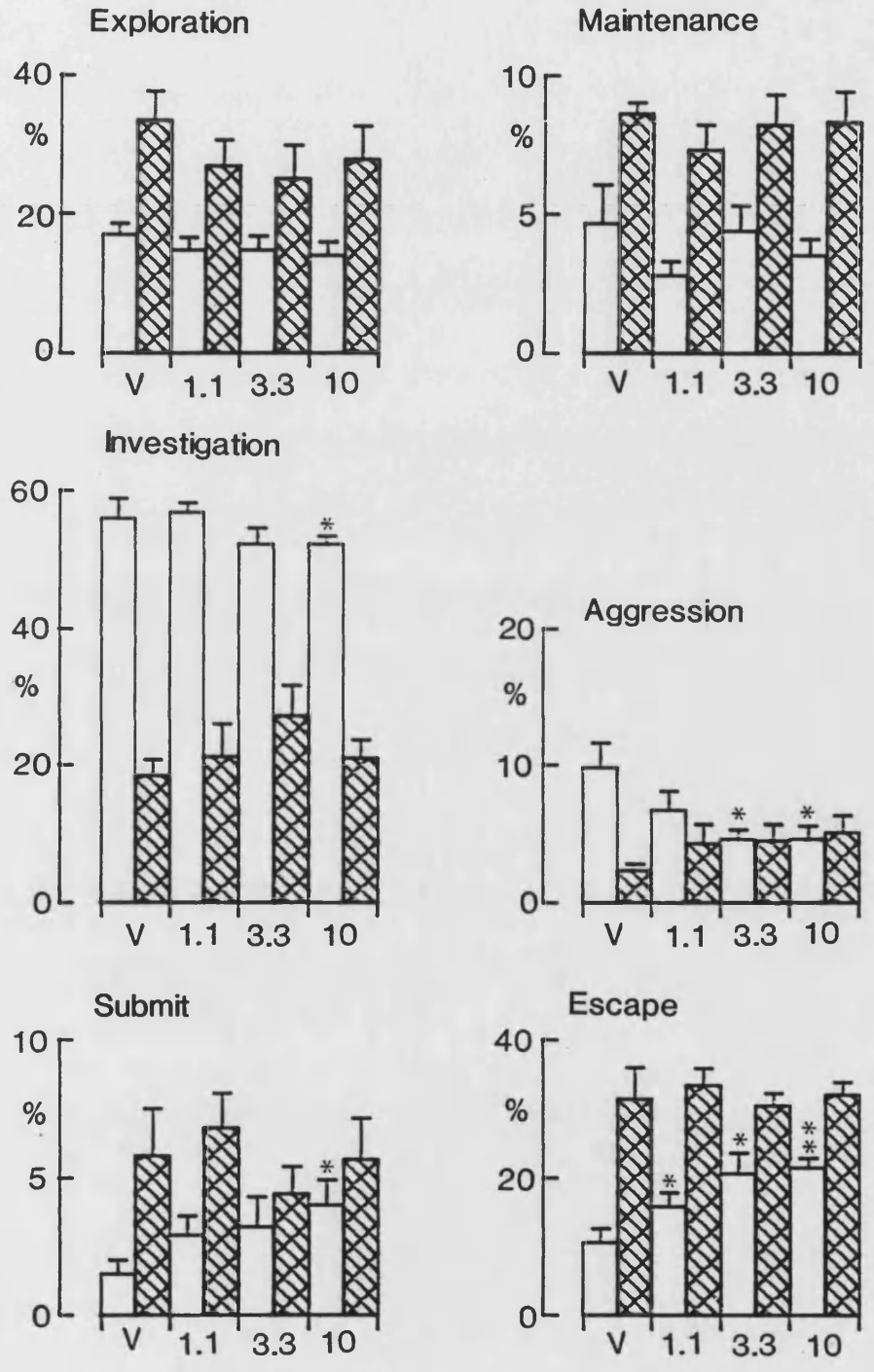
N=8 animals per group.

V, drug-vehicle (0.1M tartaric acid, 1 ml Kg^{-1} sc).

1.1, 3.3, 10, indicate fluoxetine doses (umol Kg^{-1} sc).

Pre-treatment time=30 min.

MWUT : * $p<0.05$, ** $p<0.01$ compared to respective vehicle treatment.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rats following treatment of the latter with fluoxetine, 1.1-10 umol Kg^{-1} sc.

		Fluoxetine umol Kg^{-1} sc.		
	Tartrate	1.1	3.3	10
Resident	491±47	574±33	538±25	594±31
Intruder	446±33	475±26	483±32	459±32

Table 5.5 Effect of acute fluoxetine on the total number of behaviours exhibited by resident and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

MWUT : All values not significantly different from respective vehicle treatment.

5.5.2.3 Iprindole

The behavioural profiles exhibited by resident rats following 30 minutes subcutaneous pre-treatment with H_2O, 1ml Kg^{-1}, or iprindole, 1-9 umol Kg^{-1}, and by non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.6 and Table 5.6 respectively.

5.5.2.3.1 Resident animals

Iprindole, 1-9 umol Kg^{-1} sc, induced a dose-related reduction in the level of aggression (ID_{50} = 3.82 (2.50,6.25) umol Kg^{-1}) directed at the conspecific intruder concomitant with a dose-related increase in the level of flight-escape behaviour. At these doses iprindole also appeared to increase the level of flight-submit behaviour in a dose-related manner but no significant effect was observed at any of the doses tested. Iprindole treatment had little or no effect on the levels of environmental exploration, investigation of the conspecific intruder, maintenance behaviours nor the total number of behaviours exhibited during social interaction.

5.5.2.3.2 Intruder animals

Only the level of flight-submit behaviour exhibited by intruder rats was progressively reduced during social interaction with resident rats treated with iprindole, 1-9 umol Kg^{-1} sc., although no significant differences from control levels were observed. No significant change in any of the other motivational categories was observed for intruder rats during social interaction with resident rats treated with iprindole, 1-9 umol Kg^{-1} sc.

Figure 5.6 Effect of acute treatment with iprindole on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats.

Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

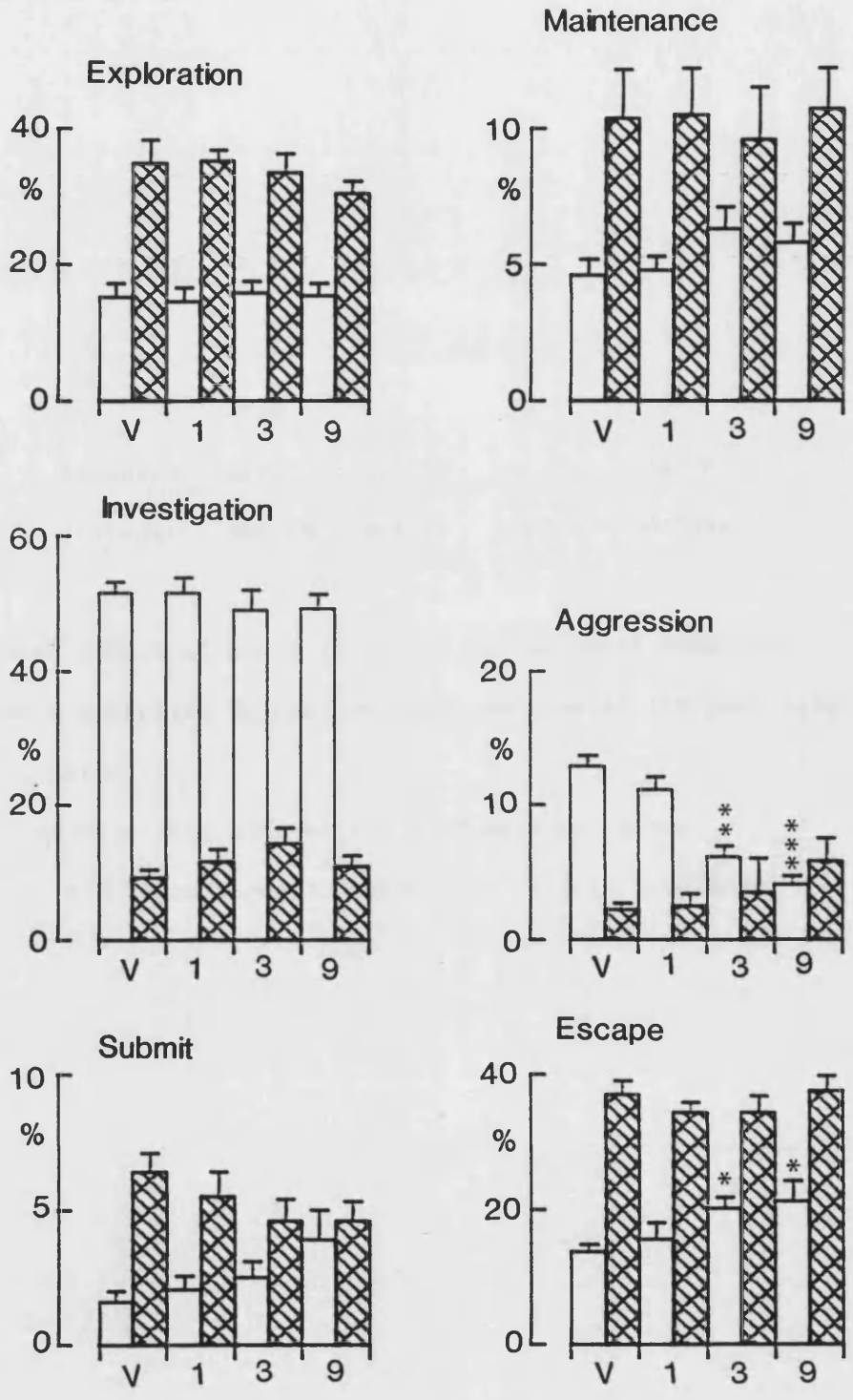
N=8 animals per group.

V, drug-vehicle (H_2O, 1 ml Kg^{-1} sc).

1, 3, 9, indicate iprindole doses (umol Kg^{-1} sc).

Pre-treatment time=30 min.

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to respective vehicle treatment.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The total number of behaviours exhibited by intruder rats were only significantly reduced during social interaction with resident rats treated with iprindole, 9 umol Kg^{-1} sc.

	Iprindole umol Kg^{-1} sc.			
	H_2O	1	3	9
Resident	664±31	657±35	697±73	686±75
Intruder	499±29	489±24	442±39	381±39a

Table 5.6 Effect of acute iprindole on the total number of behaviours exhibited by resident and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

MWUT : a, $p<0.05$ compared to respective vehicle treatment.

5.5.2.4 Mianserin

The behavioural profiles exhibited by resident rats following 30 minutes subcutaneous pre-treatment with H_2O, 1ml Kg^{-1}, or mianserin, 0.33-3 umol Kg^{-1}, and by non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.7 and Table 5.7 respectively.

5.5.2.4.1 Resident animals

Mianserin, 0.33-3 umol Kg^{-1} sc., induced a dose-related decrease in the level of aggressive behaviour (ID_{50} = 1.19 (0.16,17.4) umol Kg^{-1}) directed at the conspecific intruder concomitant with a dose-related increase in the level of flight-escape behaviour. At the same doses mianserin induced slight increases in the level of flight-submit behaviour although none reached significance nor appeared to be related to dose. Mianserin had no significant effect on environmental exploration, investigation of the conspecific intruder, maintenance behaviour nor the total number of behaviours observed during social interaction.

5.5.2.4.2 Intruder animals

Intruder rats exhibited no significant change in the level of any of the motivational categories during social interaction with resident rats treated with mianserin at any of the doses tested, even though flight-submit behaviour was slightly lower during social interaction with resident rats treated with mianserin, 1 and 3 umol Kg^{-1} sc. The total number of behaviours exhibited by intruder rats were significantly lower during social interaction with resident rats

Figure 5.7 Effect of acute treatment with mianserin on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats.

Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

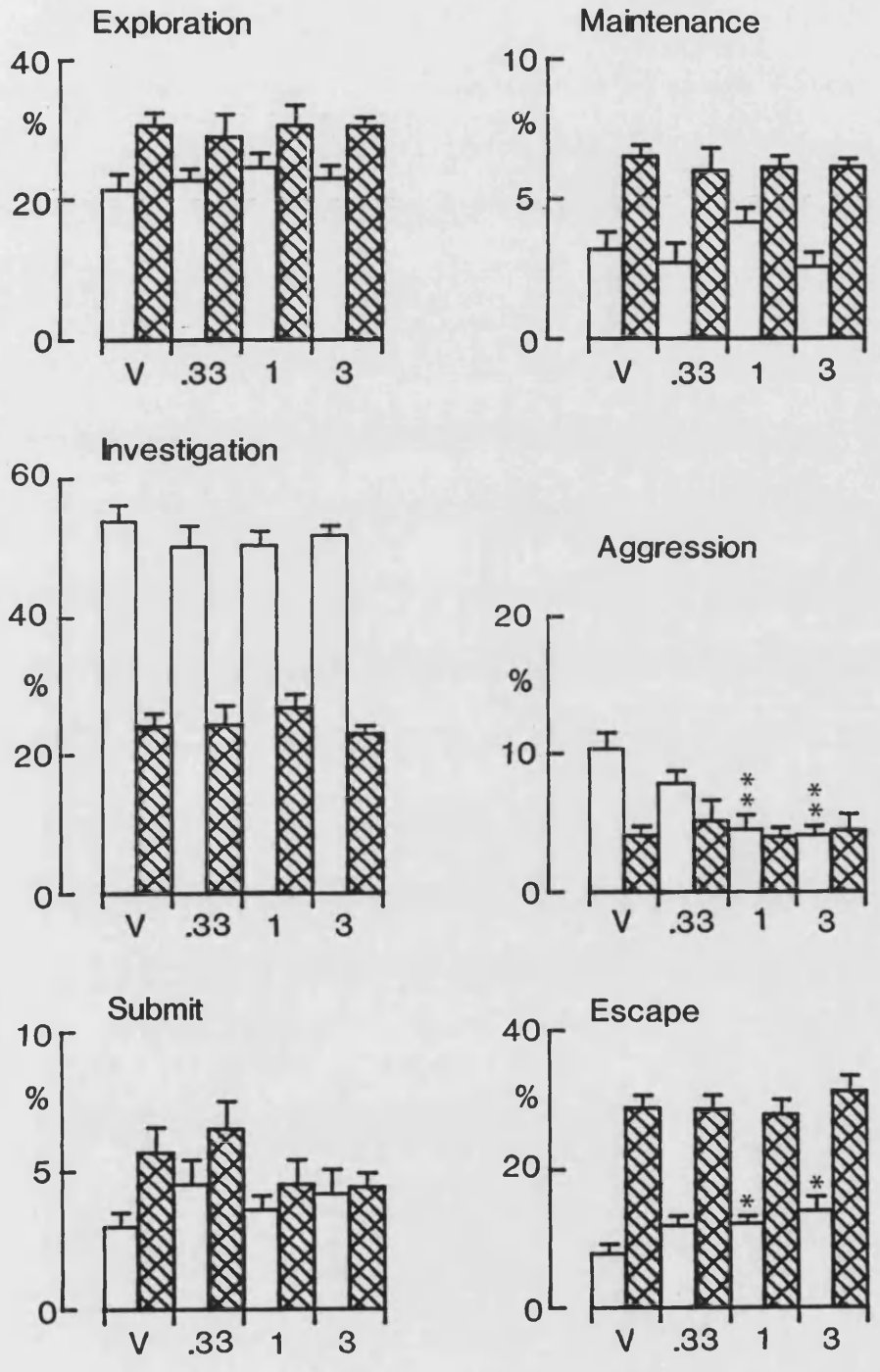
N=8 animals per group.

V, drug-vehicle (H_2O, 1 ml Kg^{-1} sc).

.33, 1, 3, indicate mianserin doses (umol Kg^{-1} sc).

Pre-treatment time=30 min.

MWUT : * $p<0.05$, ** $p<0.01$ compared to respective vehicle treatment.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treated with mianserin, 1 umol Kg^{-1} sc., but not during social interaction with resident rats treated with mianserin, 0.33 or 3 umol Kg^{-1} sc.

	Mianserin umol Kg^{-1} sc.			
	H_2O	0.33	1	3
Resident	487±22	516±34	487±12	520±27
Intruder	507±18	498±22	449±18a	498±48

Table 5.7 Effect of acute mianserin on the total number of behaviours exhibited by resident and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

MWUT : a, $p<0.05$ compared to respective vehicle treatment.

5.5.2.5 Phenelzine

The behavioural profiles exhibited by resident rats following 30 minutes subcutaneous pre-treatment with H_2O, 1ml Kg^{-1}, or phenelzine, 1-9 umol Kg^{-1}, and by non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.8 and Table 5.8 respectively.

5.5.2.5.1 Resident animals

Phenelzine, 1-9 umol Kg^{-1} sc., induced a dose-related decrease in the level of aggression (ID_{50} = 5.69 (1.41,221.5) umol Kg^{-1}) directed at the conspecific intruder concomitant with increases in both flight-escape and flight-submit behaviours (which appeared to be dose-related) although the latter failed to show any significant differences from the control level following treatment with any of the doses of phenelzine tested. Investigation of the conspecific intruder was slightly, albeit significantly, reduced following treatment with phenelzine, 3 umol Kg^{-1} sc., but not 1 or 9 umol Kg^{-1} sc. Phenelzine treatment, at any of the doses tested, failed to change the levels of the other motivational categories during social interaction or the total number of behaviours observed.

5.5.2.5.2 Intruder animals

Flight-escape behaviour exhibited by intruder rats was only significantly reduced during social interaction with resident rats treated with phenelzine, 3 umol Kg^{-1} sc. Flight-submit behaviour was slightly reduced during social interaction with resident rats treated with phenelzine, 1-9 umol Kg^{-1} sc., and the reduction in this behaviour appeared to be related to the dose administered to the resident rats, however none of the observed levels of this

Figure 5.8 Effect of acute treatment with phenelzine on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats.

Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

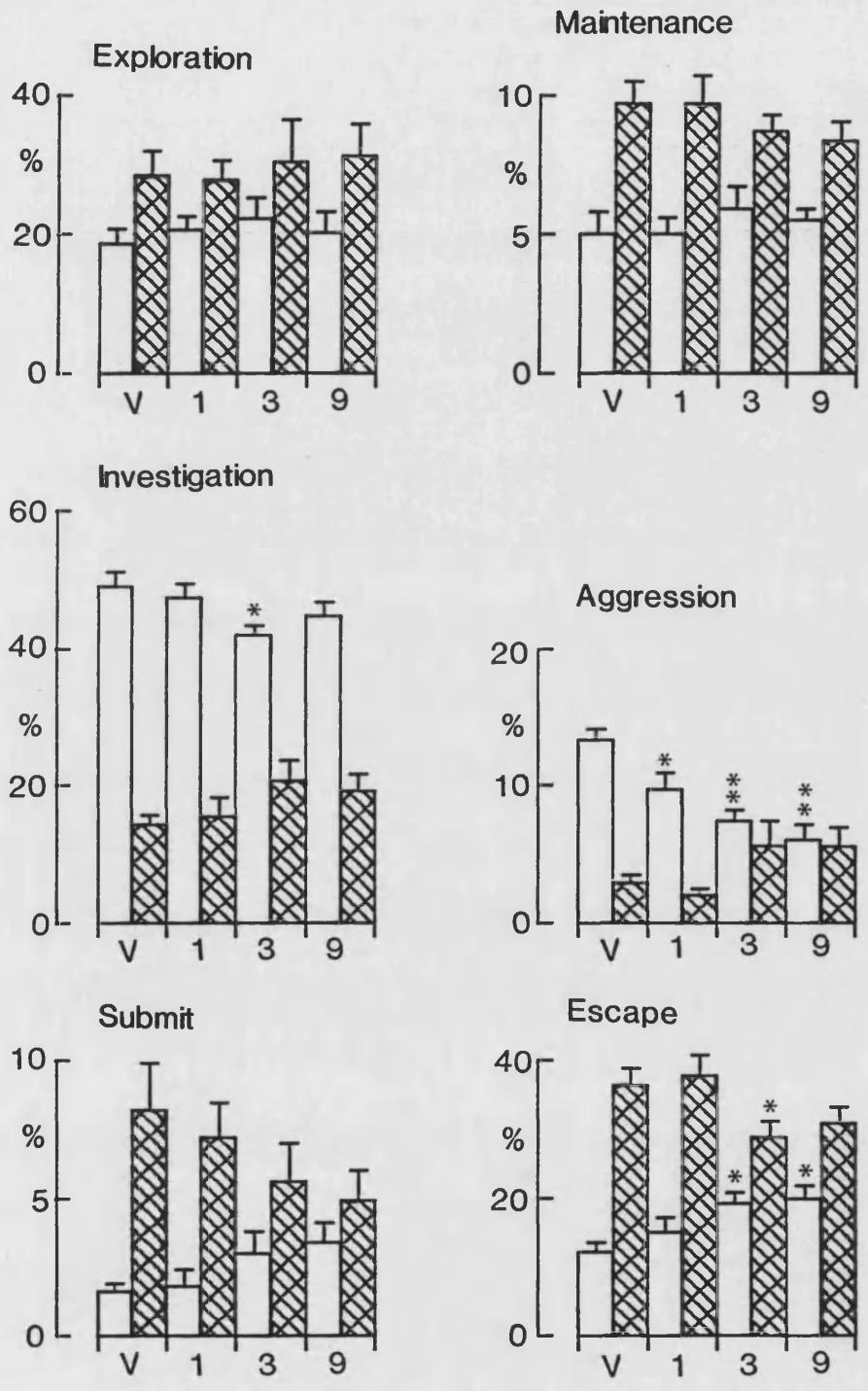
N=8 animals per group.

V, drug-vehicle (H_2O, 1 ml Kg^{-1} sc).

1, 3, 9, indicate phenelzine doses (umol Kg^{-1} sc).

Pre-treatment time=30 min.

MWUT : * $p<0.05$, ** $p<0.01$ compared to respective vehicle treatment.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motivational category were significantly different from control. Intruder rats showed little or no change in the level of environmental exploration, investigation of the conspecific resident, maintenance behaviour or the total number of behaviours observed during social interaction with resident rats treated with phenelzine at any of the doses tested.

	Phenelzine umol Kg^{-1} sc.			
	H_2O	1	3	9
Resident	675±59	680±48	645±64	717±44
Intruder	492±34	476±21	482±35	480±25

Table 5.8 Effect of acute phenelzine on the total number of behaviours exhibited by resident and non-treated intruder rats during social interaction.
Values indicate mean and sem for 8 animals per group.
MWUT : All values not significantly different from respective vehicle treatment.

5.5.2.6 Haloperidol

The behavioural profiles exhibited by resident rats following 60 minutes subcutaneous pre-treatment with 0.1M tartaric acid, 1ml Kg^{-1}, or haloperidol, 0.11-1 umol Kg^{-1}, and by non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.9 and Table 5.9 respectively.

5.5.2.6.1 Resident animals

Haloperidol, 0.11-1 umol Kg^{-1} sc., induced a dose-related reduction in both aggressive behaviour (ID_{50} = 0.19 (0.09,0.33) umol Kg^{-1}) directed at the conspecific intruder and the level of flight-submit behaviour (ID_{50} = 0.17 (0.003,0.75) umol Kg^{-1}). Haloperidol, 0.33 and 1 umol Kg^{-1} sc., induced a significant increase in both flight-escape and maintenance behaviours, although only the former appeared to be related to dose. Haloperidol, 0.11-1 umol Kg^{-1} sc., had little or no consistent or significant effect on either the level of environmental exploration or investigation of the conspecific intruder, however those same doses of haloperidol significantly and dose-relatedly reduced the total number of behaviours (ID_{50} = 0.18 (0.09,0.29) umol Kg^{-1}) observed during social interaction.

5.5.2.6.2 Intruder animals

The behavioural profile of non-drugged intruder rats was observed to change quite markedly in response to the haloperidol-induced changes in the behavioural profile of resident rats. Thus aggressive, flight-escape and flight-submit behaviours were all observed to be markedly reduced, while conversely the levels of environmental exploration and maintenance behaviours were both markedly increased, during social interaction with resident rats treated with

Figure 5.9 Effect of acute treatment with haloperidol on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

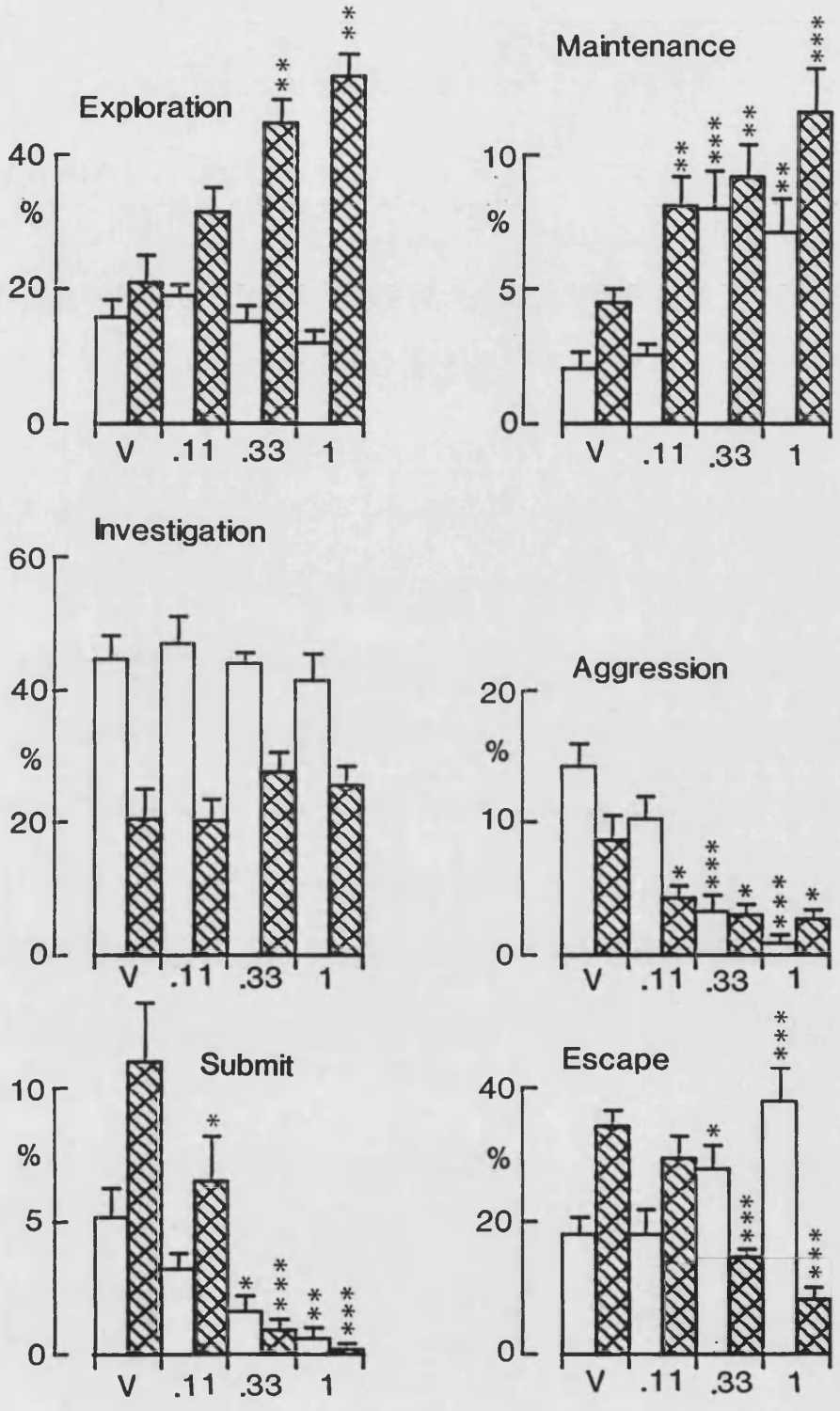
N=8 animals per group.

V, drug-vehicle (0.1M tartaric acid, 1 ml Kg^{-1} sc).

.11, .33, 1, indicate haloperidol doses (umol Kg^{-1} sc).

Pre-treatment time=60 min.

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to respective vehicle treatment.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haloperidol, 0.11-1 umol Kg^{-1} sc.; effects which in all cases appeared to be related to the dose of haloperidol received by the resident rats. No variation was observed in the level of investigation of the conspecific resident exhibited by intruder rats regardless of the treatments received by the resident rats. The total number of behaviours exhibited by intruder rats was only significantly reduced during social interaction with resident rats treated with haloperidol, 1 umol Kg^{-1} sc.

		Haloperidol umol Kg^{-1} sc.		
	Tartrate	0.11	0.33	1
Resident	697±47	466±55b	214±33c	126±23c
Intruder	489±26	485±36	408±37	349±24c

Table 5.9 Effect of acute haloperidol on the total number of behaviours exhibited by resident and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

MWUT : b, $p<0.01$; c, $p<0.001$ compared to respective vehicle treatment.

5.5.2.7 Diazepam

The behavioural profiles exhibited by resident rats following 30 minutes subcutaneous pre-treatment with diazepam, 3.3-30 umol Kg^{-1}, or vehicle, 1ml Kg^{-1} (see section 4.6 for description), and by non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.10 and Table 5.10 respectively.

5.5.2.7.1 Resident animals

Diazepam, 3.3-30 umol Kg^{-1} sc., induced a dose related increase in flight-escape and, possibly, flight-submit behaviours, although the latter were not significantly different from control. The same doses of diazepam decreased exploratory behaviour (ID_{50} = 10 umol Kg^{-1} approximately) and increased maintenance behaviour, although the effects seen following 30 umol Kg^{-1} sc. showed little or no difference from those observed following 10 umol Kg^{-1} sc. Diazepam, 10 and 30 umol Kg^{-1} sc., induced a dose-related reduction in the levels of both investigation (ID_{50} = 92.8 (33.7,2133) umol Kg^{-1}) and, more markedly, aggression (ID_{50} = 8.73 (6.03,12.3) umol Kg^{-1}) directed towards the conspecific intruder. Diazepam, 3.3-30 umol Kg^{-1} sc., induced a dose-related reduction in the total number of behaviours (ID_{50} = 7.26 (5.05,9.96) umol Kg^{-1}) observed during social interaction.

5.5.2.7.2 Intruder animals

Intruder rats exhibited increased exploratory activity and decreased flight-escape and flight-submit behaviours during social interaction which appeared to be broadly related to the dose of diazepam administered to the resident rats. No significant change in the

Figure 5.10 Effect of acute treatment with diazepam on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

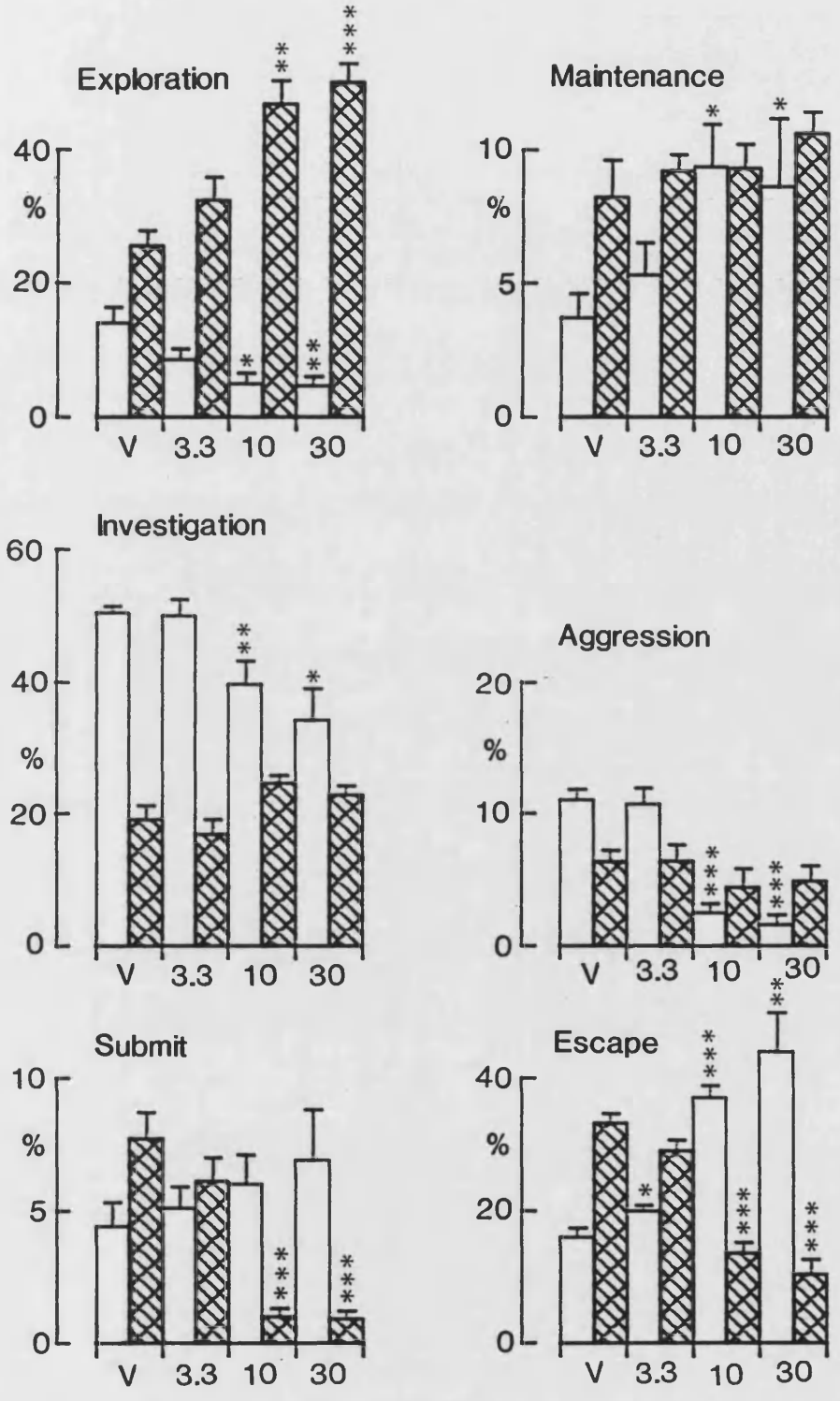
N=8 animals per group.

V, drug-vehicle (1 ml Kg^{-1} sc), see section 4.6 for description.

3.3, 10, 30, indicate diazepam doses (umol Kg^{-1} sc).

Pre-treatment time=30 min.

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to respective vehicle treatment.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level of investigation or aggressive behaviour directed at the conspecific resident rats, or in the level of maintenance behaviour exhibited by non-drugged intruder rats, was observed during social interaction regardless of the dose of diazepam administered to resident rats. The total number of behaviours exhibited by intruder rats were significantly reduced during social interaction with resident rats treated with diazepam, 10 and 30 umol Kg^{-1} sc. in a manner related to the dose of diazepam administered to the resident rats.

	Diazepam umol Kg^{-1} sc.			
	Vehicle	3.3	10	30
Resident	680±39	517±30a	199±38c	145±27c
Intruder	493±23	532±32	394±24a	346±20c

Table 5.10 Effect of acute diazepam on the total number of behaviours exhibited by resident and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

MWUT : a, $p<0.05$; c, $p<0.001$ compared to respective vehicle treatment.

Table 5.11 summarizes the minimum-effective doses of the compounds examined on the behavioural profile of resident rats together with the reason for its choice. Each of the identified doses was then administered daily to resident rats to examine the effect of chronic drug administration on the behavioural profile exhibited by resident rats during social interaction. These results, together with the effects of chronic administration of the relevant drug-vehicles, are described in section 5.5.3.

Drug	umol Kg^{-1}	Reason for choice
Clomipramine	10.0	Reduced aggressive behaviour
Fluoxetine	1.1	Reduced aggressive behaviour
Iprindole	3.0	Reduced aggressive behaviour
Mianserin	0.33	Reduced aggressive behaviour
Phenelzine	1.0	Reduced aggressive behaviour
Haloperidol	0.11	Reduced aggressive behaviour, flight-submit behaviour and total number of behaviours exhibited
Diazepam	3.3	Reduced exploration and total number of behaviours exhibited; increased flight-escape behaviour

Table 5.11 Minimum-effective doses on rodent social behaviour

5.5.3 Effect of chronic (14 day) drug or vehicle treatment on the social interaction behaviour of resident and non-drugged intruder rats

5.5.3.1 H_2O

The behavioural profiles exhibited by resident rats treated chronically with H_2O, and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.11 and Table 5.12 respectively. This group was used to control those experiments involving chronic treatment with clomipramine, iprindole, mianserin and phenelzine.

5.5.3.1.1 Resident animals

Chronic treatment with H_2O had no effect on the levels of environmental exploration, maintenance behaviour, investigation or aggression directed at the conspecific intruder. Flight-submit behaviour showed a slight, non-significant, increase from 3.7±0.8% at pre-treatment to 6.8±1.5% and 5.5±1.2% at days 7 and 14 of H_2O treatment respectively, while flight-escape behaviour was slightly, but significantly, increased from 7.1±1.6% at pre-treatment to 13.2±1.7% and 10.9±1.3% at days 7 and 14 of H_2O treatment respectively, $p<0.05$ in both cases. Following the cessation of H_2O treatment no significant change in the levels of exploratory, investigatory, aggressive or maintenance behaviours were observed although a further increase in flight-submit behaviour (to 8.8±1.9%, $p<0.05$ compared to pre-treatment level) was observed. Flight-escape behaviour at 7 days post-treatment was still significantly elevated compared to pre-treatment levels. The total number of behaviours exhibited by resident rats during social interaction was not significantly different from pre-treatment levels either during

treatment or at 7 days post-treatment.

5.5.3.1.2 Intruder animals

Untreated intruder rats demonstrated no variability in the observed levels of aggression directed at the treated resident rats, flight-submit or flight-escape behaviours either during treatment or at 7 days following the cessation of treatment of resident rats with H_2O. During the course of the experiment, intruder rats showed reduced environmental exploration and maintenance behaviour, and increased investigation of the treated resident rats together with a slight, but non-significant, increase in the total number of behaviours exhibited during social interaction; these changes, however, do not appear to be related to the period of H_2O treatment of the resident rats.

Figure 5.11 Effect of chronic treatment with H_2O on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats.

Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 9.678 uL day^{-1} <u>in vivo</u>

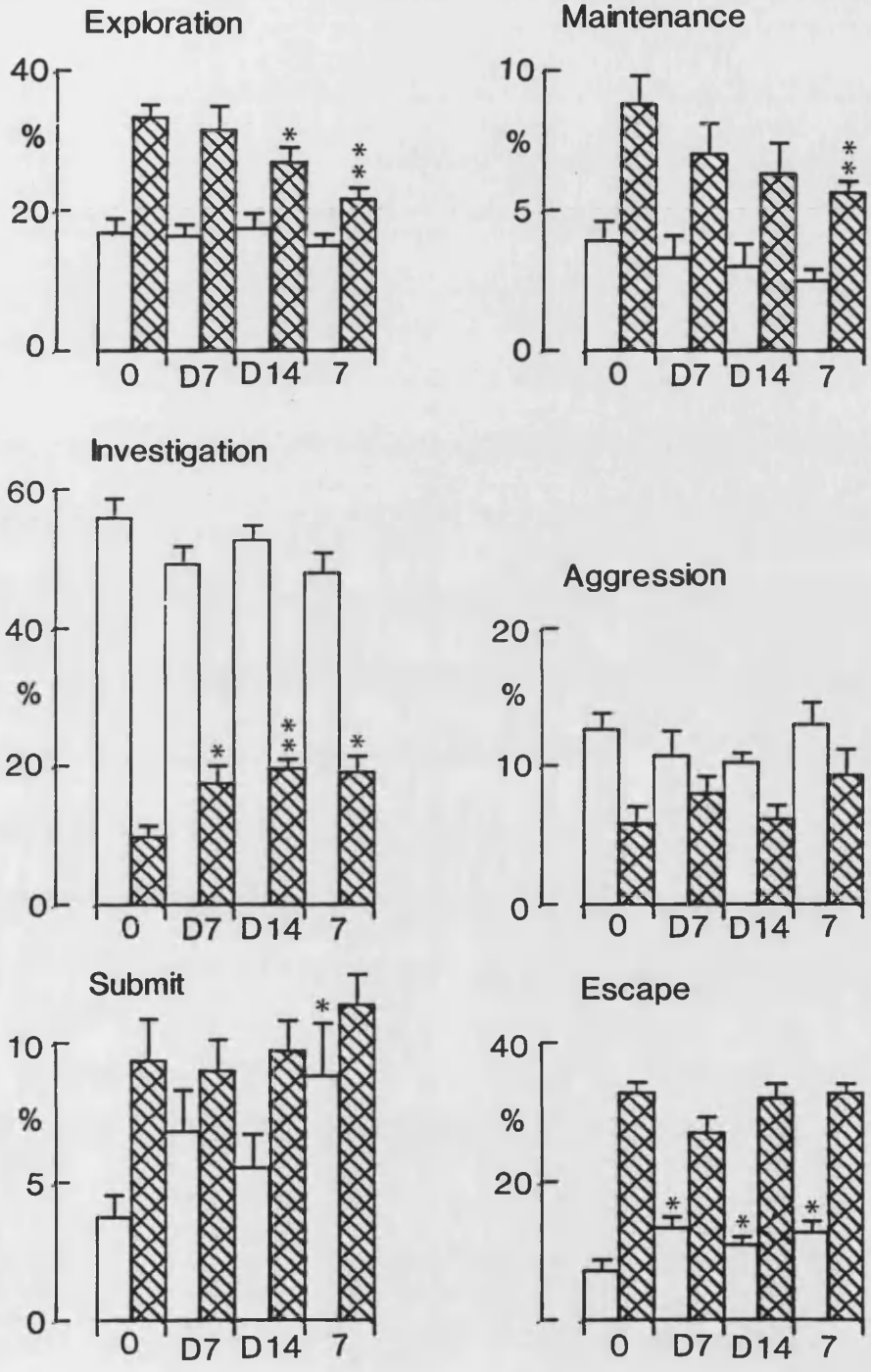
0 : Pre-treatment

D7 : 7 days treatment

D14 : 14 days treatment

7 : 7 days post-treatment

MWUT. * $p<0.05$, ** $p<0.01$ compared to pre-treatment (day 0).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	Treatment days			
Group	0	D7	D14	7
Resident	483±33	532±36	539±36	537±36
Intruder	457±27	521±33	517±25	521±21

Table 5.12 The total number of behaviours exhibited by resident treated chronically with subcutaneous H_2O, and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

Pump rate; 9.678 uL day^{-1} in vivo

0 : Pre-treatment

D7 : 7 days treatment

D14 : 14 days treatment

7 : 7 days post-treatment

MWUT : All values not significant from respective pre-treatment score.

5.5.3.2 Clomipramine

The behavioural profiles exhibited by resident rats treated chronically with clomipramine, target dose 10 umol Kg^{-1} day^{-1} sc., and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.12 and Table 5.13 respectively.

5.5.3.2.1 Resident animals

Resident rats treated chronically with clomipramine, target dose 10 umol Kg^{-1} day^{-1} sc., showed no change in the levels of investigation of the conspecific or flight-escape behaviour at 7 days of drug treatment. At both 7 and 14 days of clomipramine treatment, however, resident rats demonstrated a marked increase in the level of aggression directed at the conspecific intruder, from 12.2±1.7% at pre-treatment to 20.1±3.1% ($p<0.05$) and 23.0±1.7% ($p<0.01$) at days 7 and 14 of clomipramine treatment respectively, concomitant with a slight increase in flight-submit behaviour from 2.2±0.8% at pre-treatment to 3.9±0.6% ($p<0.05$) and 4.2±1.1% (not significant) at days 7 and 14 of treatment respectively, and significant decreases in both environmental exploration and maintenance behaviour at 14 days of treatment; from 23.4±3.0% and 3.8±0.6% at pre-treatment to 13.3±1.0% and 2.2±0.5% respectively ($p<0.05$ in both cases). Seven days after the end of clomipramine treatment the levels of aggression directed at the conspecific intruder and maintenance behaviour had both returned to the relative pre-treatment levels concomitant with a marked and significant increase in flight-escape behaviour (from 7.3±1.1% at pre-treatment and 7.5±1.1% at day 14 of treatment to 14.9±1.8% at 7 days post-treatment, $p<0.01$ and $p<0.001$ respectively), and a further

slight increase in flight-submit behaviour. Resident rats exhibited an increase in the total number of behaviours during social interaction at 7 (not significant) and 14 days of clomipramine treatment; this increase, although not significant, was still observed at 7 days following the cessation of drug treatment.

5.5.3.2.2 Intruder animals

During the period of clomipramine treatment of the resident rats, non-treated intruder animals showed a significant increase in the level of flight-submit behaviour (from 8.3±1.7% at pre-treatment to 14.7±2.1% and 14.44±1.5% after 7 and 14 days of treatment respectively, $p<0.05$ in both cases), which partly returned to the control level (10.7±2.0%, not significant from pre-treatment level) at 7 days after treatment of the resident rats, but not in the level of flight-escape behaviour. In addition, intruder rats showed reduced maintenance behaviour during the period of drug treatment of the resident rats, but no change in the level of investigatory behaviour directed at the conspecific resident rats. 7 days after clomipramine treatment of the resident rats, however, the intruder rats showed a significant elevation in the level of investigation directed at the resident rats; from 15.2±1.5% at pre-treatment to 24.6±3.7% ($p<0.05$). Throughout the period of the experiment intruder rats exhibited a progressive decrease in the level of environmental exploration in addition to increases in the total number of behaviours observed.

Figure 5.12 Effect of chronic treatment with clomipramine, target dose 10 umol Kg^{-1} day^{-1}, on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction. Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 9.678 uL day^{-1} <u>in vivo</u>

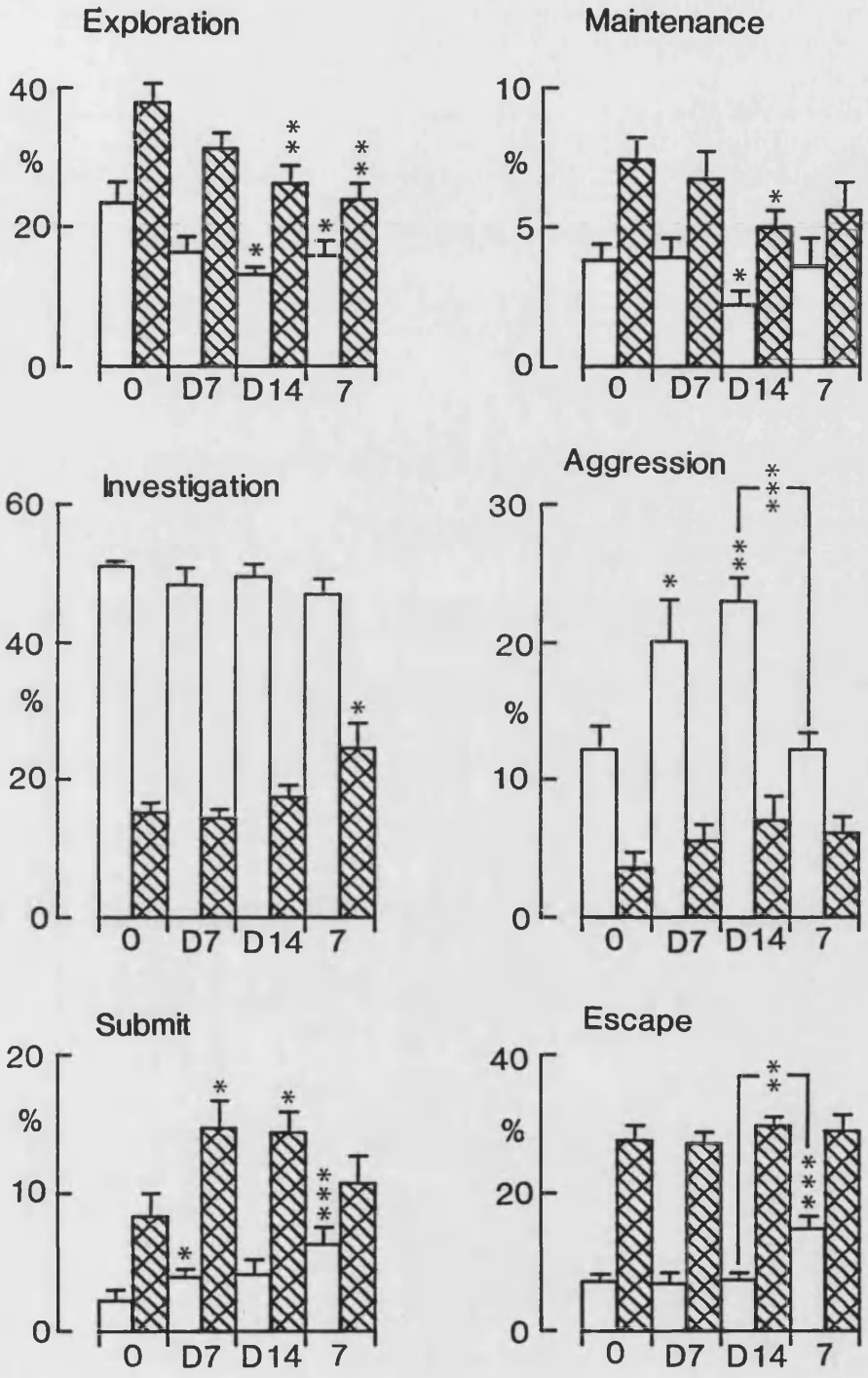
0 : Pre-treatment Clomipramine dose; 10.82±0.05 umol Kg^{-1}

D7 : 7 days treatment Clomipramine dose; 10.26±0.11 umol Kg^{-1}

D14 : 14 days treatment Clomipramine dose; 9.64±0.14 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to pre-treatment (day 0) except where indicated.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	Treatment days			
Group	0	D7	D14	7
Resident	402±34	473±29	504±26a	489±38
Intruder	418±29	483±26	511±38a	506±29

Table 5.13 The total number of behaviours exhibited by resident rats treated chronically with clomipramine, target dose 10 umol Kg^{-1} day^{-1} sc., and non-treated intruder rats during social interaction. Values indicate mean and sem for 8 animals per group.

Pump rate; 9.678 uL day^{-1} in vivo

0 : Pre-treatment Clomipramine dose; 10.82±0.05 umol Kg^{-1}

D7 : 7 days treatment Clomipramine dose; 10.26±0.11 umol Kg^{-1}

D14 : 14 days treatment Clomipramine dose; 9.64±0.14 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : a, $p<0.05$. All other values not significant from respective pre-treatment score.

5.5.3.3 Iprindole

The behavioural profiles exhibited by resident rats treated chronically with iprindole, target dose 3 umol Kg^{-1} day^{-1} sc., and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.13 and Table 5.14 respectively.

5.5.3.3.1 Resident animals

Resident rats treated chronically with iprindole, target dose 3 umol Kg^{-1} day^{-1} sc., exhibited a marked increase in the level of aggression directed at the conspecific intruder from 7.0±0.9% at pre-treatment to 16.8±0.8% ($p<0.001$) and 16.2±1.6% ($p<0.01$) at 7 and 14 days of drug treatment respectively. Although no significant change was observed in any of the other motivational categories monitored, the data suggest slight reductions in both maintenance behaviour and investigation of the conspecific intruder, together with a slight, non-significant, increase in flight-submit behaviour. By 7 days following the cessation of drug treatment the level of aggression had returned to the pre-treatment level concomitant with significant increases in both flight-submit and flight-escape behaviours; from 1.6±0.4% and 12.6±1.4% at day 14 of drug treatment to 3.3±0.5% and 17.6±1.4% respectively, $p<0.05$ in both cases. Resident rats showed an increase, albeit not significant, in the total number of behaviours exhibited during social interaction at 14 days of iprindole treatment and at 7 days post-treatment.

5.5.3.3.2 Intruder animals

During the period of iprindole treatment of the resident rats, non-treated intruder rats demonstrated a marked elevation in the level of flight-submit (from 3.8±0.6% at pre-treatment to 9.2±2.4%, $p<0.05$, and 9.5±1.8%, $p<0.01$, at days 7 and 14 of treatment respectively), but not flight-escape, behaviour, concomitant with a slight reduction in both environmental exploration (which was significant at day 14 of drug treatment of the resident rats) and possibly, although not significantly, maintenance behaviour. No significant change was observed in the levels of either investigatory or aggressive behaviours exhibited by intruder rats during the period of iprindole treatment of the resident rats. 7 days after drug treatment of the resident rats, the intruder rats still exhibited elevated flight-submit behaviour and reduced environmental exploration, together with a significant elevation in aggressive behaviour (from 1.8±0.6% at pre-treatment to 4.6±1.0%, $p<0.05$) directed at the resident rats. Intruder rats demonstrated a slight, progressive, but not significant decrease in investigation of the conspecific resident rats throughout the duration of the experiment. The total number of behaviours exhibited by intruder rats showed no variation throughout the duration of the experiment.

Figure 5.13 Effect of chronic treatment with iprindole, target dose 3 umol Kg^{-1} day^{-1} sc., on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats.

Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 10.368 uL day^{-1} *in vivo*

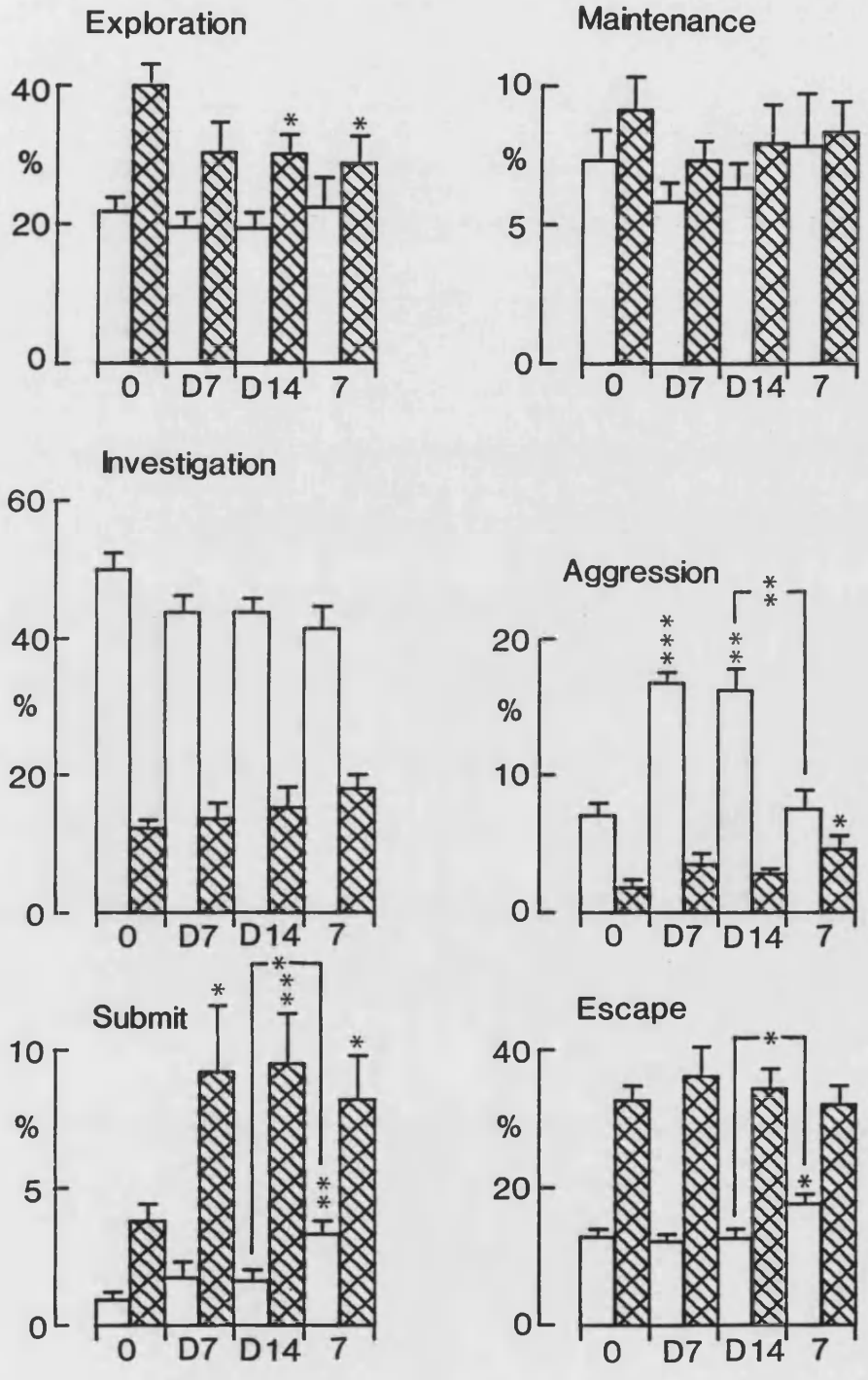
O : Pre-treatment Iprindole dose; 3.295±0.010 umol Kg^{-1}

D7 : 7 days treatment Iprindole dose; 2.994±0.019 umol Kg^{-1}

D14 : 14 days treatment Iprindole dose; 2.767±0.003 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to pre-treatment (day 0) except where indicated.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	Treatment days			
Group	0	D7	D14	7
Resident	502±49	516±36	628±56	591±67
Intruder	437±59	457±47	483±35	473±34

Table 5.14 The total number of behaviours exhibited by resident rats treated chronically with iprindole, target dose 3 umol Kg^{-1} day^{-1} sc., and non-treated intruder rats during social interaction.
Values indicate mean and sem for 8 animals per group.
Pump rate; 10.368 uL day^{-1} <u>in vivo</u>
0 : Pre-treatment Iprindole dose; 3.295±0.010 umol Kg^{-1}
D7 : 7 days treatment Iprindole dose; 2.994±0.019 umol Kg^{-1}
D14 : 14 days treatment Iprindole dose; 2.767±0.003 umol Kg^{-1}
7 : 7 days post-treatment
MWUT : All values not significant from respective pre-treatment score.

5.5.3.4 Mianserin

The behavioural profiles exhibited by resident rats treated chronically with mianserin, target dose 0.33 umol Kg^{-1} day^{-1} sc., and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.14 and Table 5.15 respectively.

5.5.3.4.1 Resident animals

Resident rats treated chronically with mianserin, target dose 0.33 umol Kg^{-1} day^{-1} sc., demonstrated a progressive and significant increase in aggressive behaviour directed at the conspecific intruder from 8.7±0.7% at pre-treatment to 16.4±1.0% and 21.6±1.5%, $p<0.001$ in both cases, at 7 and 14 days of drug treatment respectively, concomitant with progressive, significant decreases in both flight-submit and flight-escape behaviour, from 4.8±1.5% and 11.8±0.6% at pre-treatment to 1.5±0.6% ($p<0.05$) and 5.1±0.9% ($p<0.001$) by day 14 of treatment, respectively, and possibly, although not significantly, in maintenance behaviour. 7 days after drug treatment the levels of aggression directed at the conspecific intruder, flight-escape and maintenance behaviours had fully returned to their pre-treatment levels, while the mianserin-induced reduction in flight-submit behaviour only partly, although significantly compared to the level observed at day 14 of drug treatment, returned to the pre-treatment level. No significant change was observed in the levels of environmental exploration or investigation of the conspecific intruder either at 7 or 14 days of mianserin treatment or indeed at 7 days after drug treatment. The total number of behaviours exhibited by resident rats showed no variation either during the period of mianserin treatment or at 7

days post-treatment.

5.5.3.4.2 Intruder animals

During the period of mianserin treatment of the resident rats, non-treated intruder rats exhibited a progressive increase in flight-submit behaviour and a slight increase in flight-escape behaviour, from 7.3±0.9% and 28.3±1.1% at pre-treatment to 13.5±1.6% ($p<0.01$) and 35.3±2.0% ($p<0.05$) by day 14 of treatment respectively, concomitant with slight (although not significant) decreases in both maintenance behaviour and aggression directed at the conspecific resident rats. By 7 days following the cessation of drug treatment of the resident rats, the elevated level of flight-submit, and the slight reductions in both maintenance and aggressive behaviours, but not flight-escape behaviour, exhibited by intruder rats had returned either fully or partly to their respective pre-treatment levels. Throughout the duration of the experiment intruder rats demonstrated a progressive and significant decrease in environmental exploration concomitant with a progressive, albeit slight, increase in investigation of the conspecific resident rats. The total number of behaviours exhibited by intruder rats during social interaction were only slightly, but not significantly, reduced at both 14 days of mianserin treatment of the resident rats and at 7 days post-treatment.

Figure 5.14 Effect of chronic treatment with mianserin, target dose 0.33 umol Kg^{-1} day^{-1} sc., on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.
Open columns, resident rats. Hatched columns, intruder rats.
Columns indicate mean (and sem) percentage of total behaviours for each motivational category.
N=8 animals per group.
Pump rate; 9.678 uL day^{-1} <u>in vivo</u>

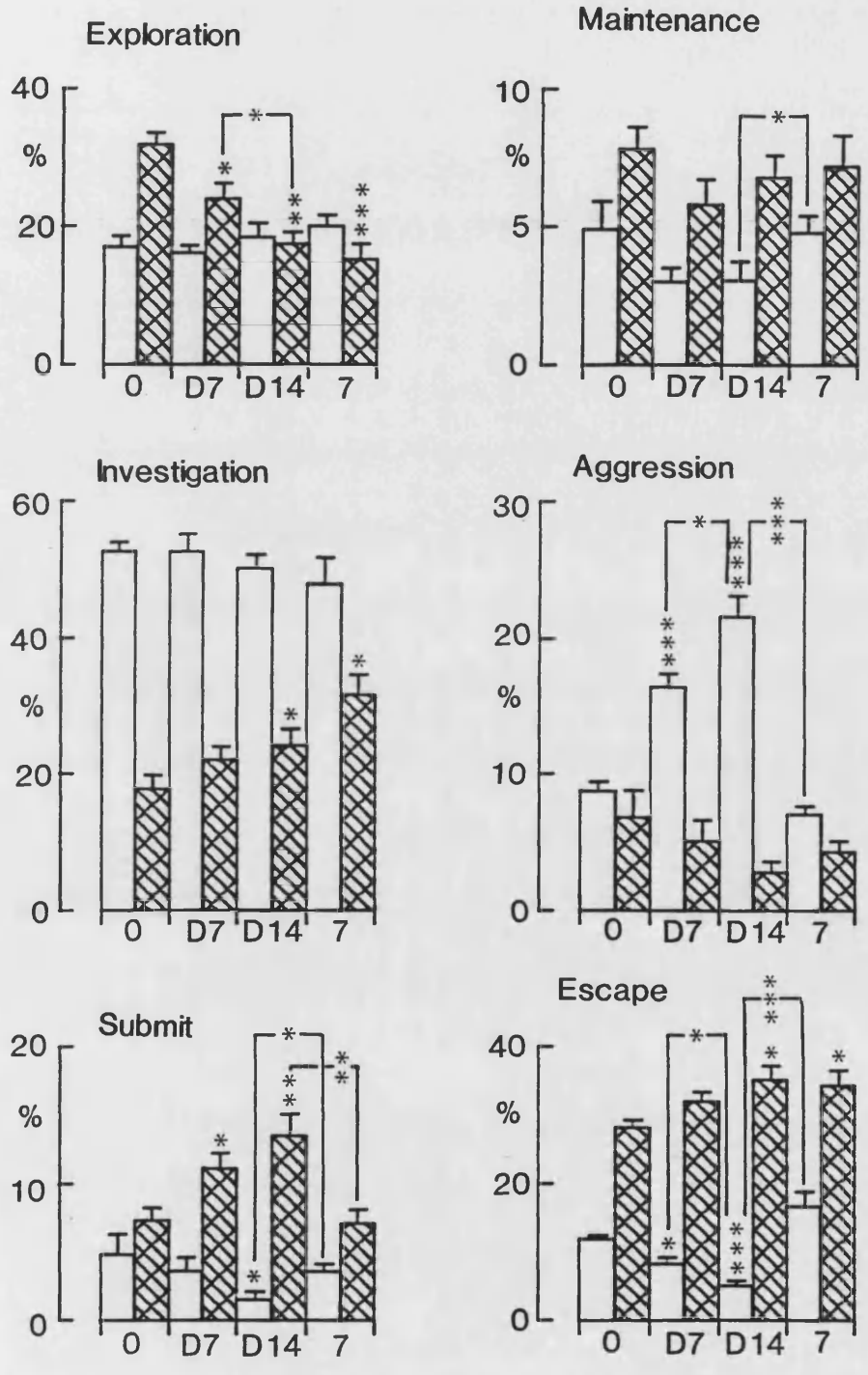
O : Pre-treatment Mianserin dose; 0.361±0.0012 umol Kg^{-1}

D7 : 7 days treatment Mianserin dose; 0.327±0.0018 umol Kg^{-1}

D14 : 14 days treatment Mianserin dose; 0.304±0.0034 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to pre-treatment (day O) except where indicated.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D7
D14
7

	Treatment days			
Group	0	D7	D14	7
Resident	509±37	509±40	500±34	500±28
Intruder	490±15	473±38	431±31	436±30

Table 5.15 The total number of behaviours exhibited by resident rats treated chronically with mianserin, target dose 0.33 umol Kg^{-1} day^{-1} sc., and non-treated intruder rats during social interaction.
Values indicate mean and sem for 8 animals per group.
Pump rate; 9.678 uL day^{-1} in vivo
0 : Pre-treatment Mianserin dose; 0.361±0.0012 umol Kg^{-1}
D7 : 7 days treatment Mianserin dose; 0.327±0.0018 umol Kg^{-1}
D14 : 14 days treatment Mianserin dose; 0.304±0.0034 umol Kg^{-1}
7 : 7 days post-treatment
MWUT : All values not significant from respective pre-treatment score.

5.5.3.5 Phenelzine

The behavioural profiles exhibited by resident rats treated chronically with phenelzine, target dose 1 umol Kg^{-1} day^{-1} sc., and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.15 and Table 5.16 respectively.

5.5.3.5.1 Resident animals

Resident rats treated chronically with phenelzine, target dose 1 umol Kg^{-1} day^{-1} sc., exhibited a marked increase in the level of aggression directed at the conspecific intruder, from 7.5±1.0% at pre-treatment to 15.6±1.6% and 17.3±1.8% ($p<0.01$ in both cases) at 7 and 14 days of drug treatment respectively, concomitant with a decrease in both maintenance and flight-escape behaviours, from 8.4±0.6% and 17.6±1.4% at pre-treatment to 5.1±0.7% ($p<0.01$) and 13.1±1.1% ($p<0.05$) at day 14 of treatment respectively. By 7 days post-treatment resident rats still exhibited a significant elevation in aggressive behaviour (12.0±1.3%; $p>0.05$ compared to the level observed prior to phenelzine treatment) although this was significantly lower than the level observed at 14 days of phenelzine treatment. Indeed the levels of aggressive and flight-escape behaviours exhibited by resident rats were not observed to return fully to their respective pre-treatment levels until 14 days after phenelzine treatment. At this time post-phenelzine treatment the level of maintenance behaviour was still slightly, although not significantly, reduced compared to the level observed prior to drug treatment. No significant change in the levels of environmental exploration, investigation of the conspecific intruder or flight-submit behaviour was observed either during or following

phenelzine treatment, although the data may suggest a slight elevation of flight-submit behaviour at day 14 after phenelzine treatment. The total number of behaviours exhibited by resident rats during social interaction were markedly raised both during and following chronic phenelzine treatment.

5.5.3.5.2 Intruder animals

During the period of phenelzine treatment of resident rats, non-treated intruder rats demonstrated increased levels in both flight-submit and flight escape behaviour, from 4.1±0.8% and 28.6±1.8% at pre-treatment to 9.6±1.4% ($p<0.05$) and 39.4±2.7% (not significant) respectively, at day 14 of treatment, concomitant with reduced levels of environmental exploration, from 40.7±2.7% at pre-treatment to 24.3±1.6% ($p<0.01$) at day 14 of treatment. At 7 days post-treatment of the resident rats, intruder rats still demonstrated elevated levels of both flight-submit and flight-escape behaviours and reduced levels of environmental exploration; indeed only the former showed any degree of reversal toward pre-treatment levels by 14 days after phenelzine treatment of the resident rats. No significant change was observed in the levels of investigation or aggression directed at the conspecific resident rats, maintenance behaviour or the total number of behaviours exhibited by intruder rats during social interaction either during or following the period of drug treatment of the resident rats.

Figure 5.15 Effect of chronic treatment with phenelzine, target dose 1 umol Kg^{-1} day^{-1} sc., on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction. Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 9.72 uL day^{-1} <u>in vivo</u>

0 : Pre-treatment Phenelzine dose; 1.098±0.006 umol Kg^{-1}

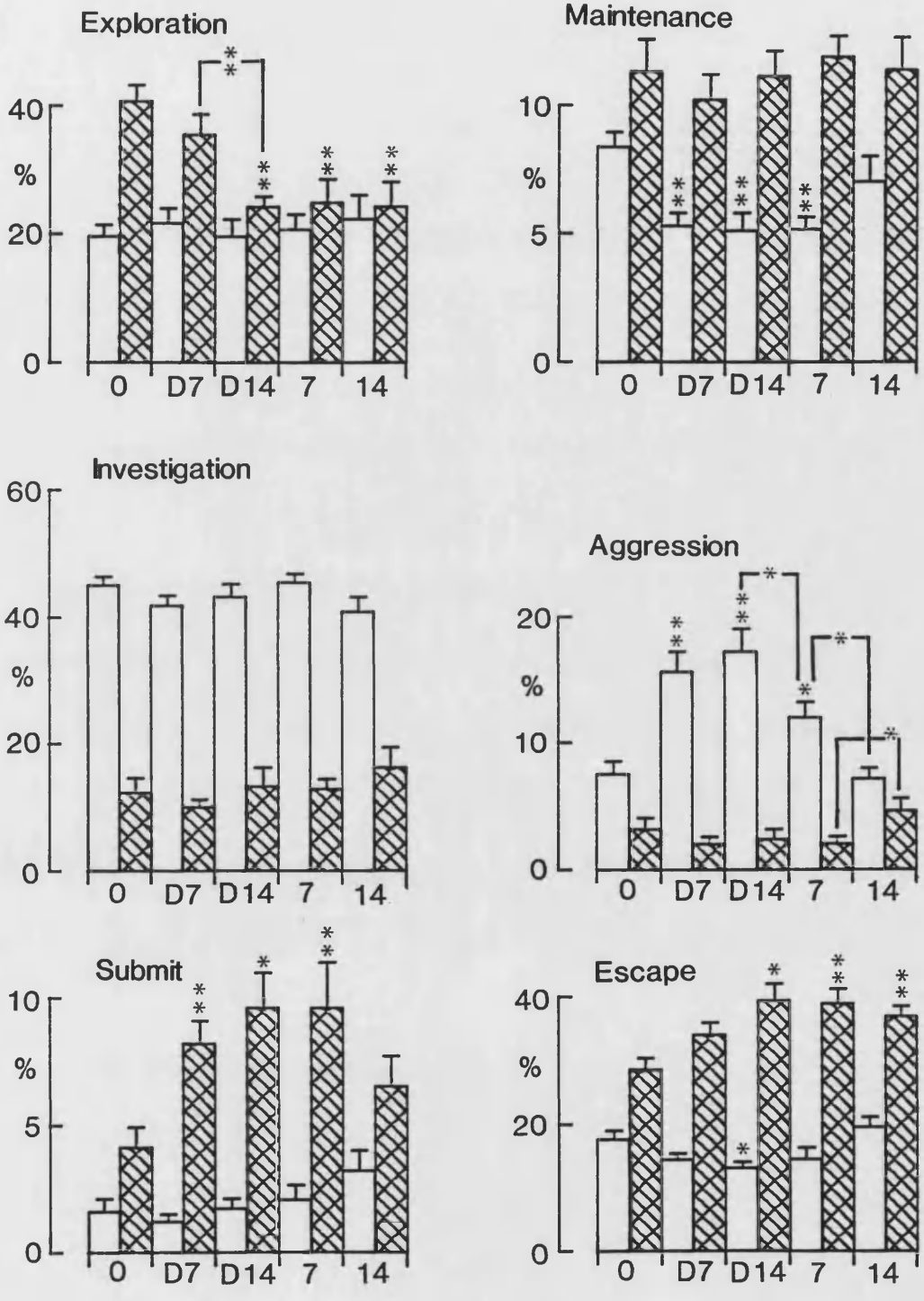
D7 : 7 days treatment Phenelzine dose; 1.004±0.009 umol Kg^{-1}

D14 : 14 days treatment Phenelzine dose; 0.909±0.010 umol Kg^{-1}

7 : 7 days post-treatment

14 : 14 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$ compared to pre-treatment (day 0) except where indicated.


Exploration
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Investigation
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%
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Aggression
20
10
Submit
Escape

	Treatment days				
Group	0	D7	D14	7	14
Resident	532±52	628±33	681±35a	777±47b	729±39a
Intruder	444±38	538±19	499±19	449±38	457±29

Table 5.16 The total number of behaviours exhibited by resident rats treated chronically with phenelzine, target dose 1 umol Kg^{-1} day^{-1} sc., and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

Pump rate; 9.72 uL day^{-1} in vivo

0 : Pre-treatment Phenelzine dose; 1.098±0.006 umol Kg^{-1}

D7 : 7 days treatment Phenelzine dose; 1.004±0.009 umol Kg^{-1}

D14 : 14 days treatment Phenelzine dose; 0.909±0.010 umol Kg^{-1}

7 : 7 days post-treatment

14 : 14 days post-treatment

MWUT : a, $p<0.05$; b, $p<0.01$. All other values not significant from respective pre-treatment score.

5.5.3.6 Tartaric acid

The behavioural profiles exhibited by resident rats treated chronically with 0.1M tartaric acid, and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.16 and Table 5.17 respectively. This experiment was designed as the control for chronic administration of fluoxetine and haloperidol.

5.5.3.6.1 Resident animals

Chronic treatment with 0.1M tartaric acid had no significant effect on the levels of environmental exploration or maintenance behaviour either during treatment or at 7 days post-treatment. Although investigation of the conspecific intruder was reduced from 54.4±1.0% at pre-treatment to 44.8±1.3% ($p<0.001$) by day 7 of treatment, no significant change from the pre-treatment level was observed at 14 days treatment or at 7 days post-treatment. Both flight-submit and flight-escape behaviours were increased from pre-treatment levels of 1.7±0.3% and 12.5±1.5% to 5.7±0.8% and 21.8±1.4% ($p<0.01$ in both cases) respectively, at day 7 of treatment, however flight-submit, but not flight-escape, behaviour returned to the pre-treatment level by day 14 of treatment. By day 7 post-treatment both flight-submit and flight-escape behaviours were slightly, but not significantly, increased compared to their respective pre-treatment levels. Aggressive behaviour directed at the conspecific intruder progressively, although not significantly, decreased thoughout the duration of the experiment and would therefore not appear to be related to the duration of chronic treatment with tartaric acid. The total number of behaviours exhibited by resident rats during social interaction were not

significantly different from pre-treatment either during tartaric acid treatment or at 7 days post-treatment.

5.5.3.6.2 Intruder animals

By 7 days of chronic treatment of the resident rats with tartaric acid, non-treated intruder rats demonstrated reduced environmental exploration from 40.8±3.6% at pre-treatment to 28.1±3.3% ($p<0.05$), which was maintained thoughout the duration of the experiment, and possibly maintenance behaviour (although not significantly) concomitant with significant increases in both investigation and aggression, from 13.3±2.1% and 2.6±0.9% at day 0 to 22.2±2.7% ($p<0.05$) and 9.6±1.1% ($p<0.01$) respectively, directed at the conspecific resident rats. By day 14 of the experiment however, the levels of investigation and aggression had returned, albeit partly in the case of investigation, to those levels observed at day 0. No significant change was observed in the level of flight-escape behaviour exhibited by intruder rats either during the period of tartaric acid treatment of resident rats or indeed at 7 days post-treatment, however flight-submit behaviour progressively (although not significantly) increased throughout the duration of the experiment.

Figure 5.16 Effect of chronic treatment with 0.1M tartaric acid on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction.

Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 9.678 (n=4) and 10.584 (n=4) uL day^{-1} in vivo

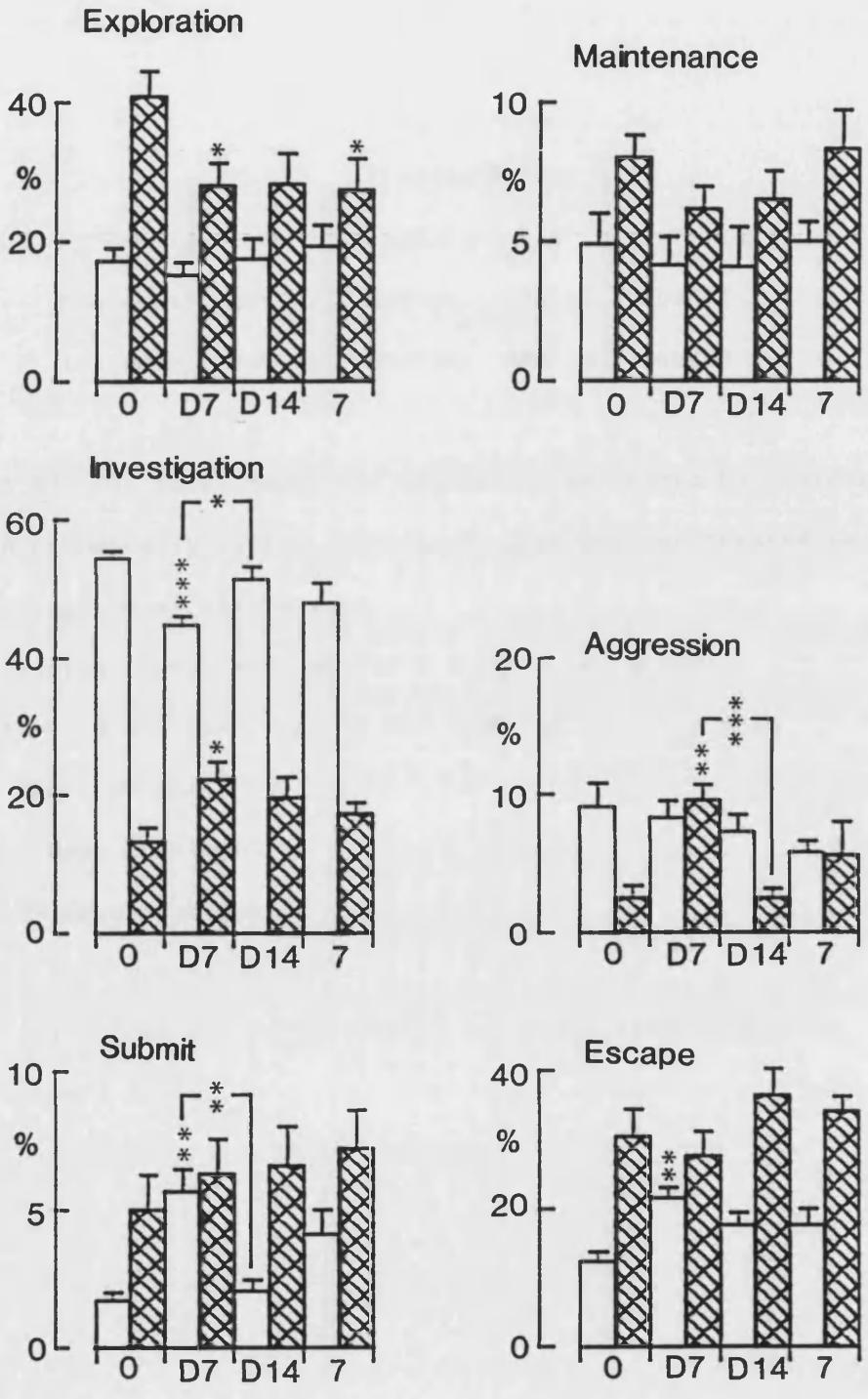
O : Pre-treatment

D7 : 7 days treatment

D14 : 14 days treatment

7 : 7 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to pre-treatment (day O) except where indicated.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	Treatment days			
	0	D7	D14	7
Resident	542±47	652±54	575±67	552±71
Intruder	485±23	488±34	440±29	455±53

Table 5.17 The total number of behaviours exhibited by resident rats treated chronically with 0.1M tartaric acid and non-treated intruder rats during social interaction.

Values indicate mean and sem for 8 animals per group.

Pump rate; 9.678 (n=4) and 10.584 (n=4) uL day^{-1} in vivo

0 : Pre-treatment

D7 : 7 days treatment

D14 : 14 days treatment

7 : 7 days post-treatment

MWUT : All values not significantly different from respective pre-treatment score.

5.5.3.7 Fluoxetine

The behavioural profiles exhibited by resident rats treated chronically with fluoxetine, target dose 1.1 umol Kg^{-1} day^{-1} sc., and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.17 and Table 5.18 respectively.

5.5.3.7.1 Resident animals

Resident rats treated chronically with fluoxetine, target dose 1.1 umol Kg^{-1} day^{-1} sc., demonstrated a marked increase in the level of aggression directed at the conspecific intruder from 8.9±0.7% at pre-treatment to 20.2±1.3% and 19.5±1.9% ($p<0.001$ in both cases) at days 7 and 14 of fluoxetine treatment respectively, concomitant with slight, but not significant, decreases in environmental exploration and maintenance behaviour (day 7 of treatment only), investigation of the conspecific intruder (day 14 of treatment only), and flight-escape behaviour (days 7 and 14 of treatment). By 7 days following drug treatment the levels of environmental exploration, maintenance behaviour, aggression and flight-escape behaviour had returned to their respective pre-treatment levels, while the level of investigation was still slightly, but not significantly, reduced, and that of flight-submit behaviour was slightly, but not significantly, increased. Resident animals exhibited an increase, although not significant, in the total number of behaviours observed at both 7 and 14 days of treatment which returned to the pre-treatment level at 7 days following the cessation of drug treatment.

5.5.3.7.2 Intruder animals

Non-treated intruder rats demonstrated little or no significant change in the levels of maintenance behaviour, investigation or aggression directed at the conspecific resident rats, flight-escape behaviour or the total number of behaviours observed during social interaction either during the period of fluoxetine treatment of the resident rats, or indeed at 7 days post-treatment, compared to their respective levels at day 0. Intruder rats exhibited increased flight-submit behaviour by day 7 of the experiment, although not significantly different from the level at day 0, which progressively returned towards the level observed at day 0 throughout the remainder of the experiment. In addition, intruder rats exhibited reduced environmental exploration from 34.6±5.6% at day 0 to 27.5±2.5% and 25.9± 3.2% (not significant in both cases) by days 7 and 14 respectively, which was further reduced to 19.6±2.6% ($p<0.05$, compared to the level observed at day 0) by day 7 after fluoxetine treatment of the resident rats.

Figure 5.17 Effect of chronic treatment with fluoxetine, target dose 1.1 umol Kg^{-1} day^{-1} sc., on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction. Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 9.678 (n=4) and 10.584 (n=4) uL day^{-1} in vivo

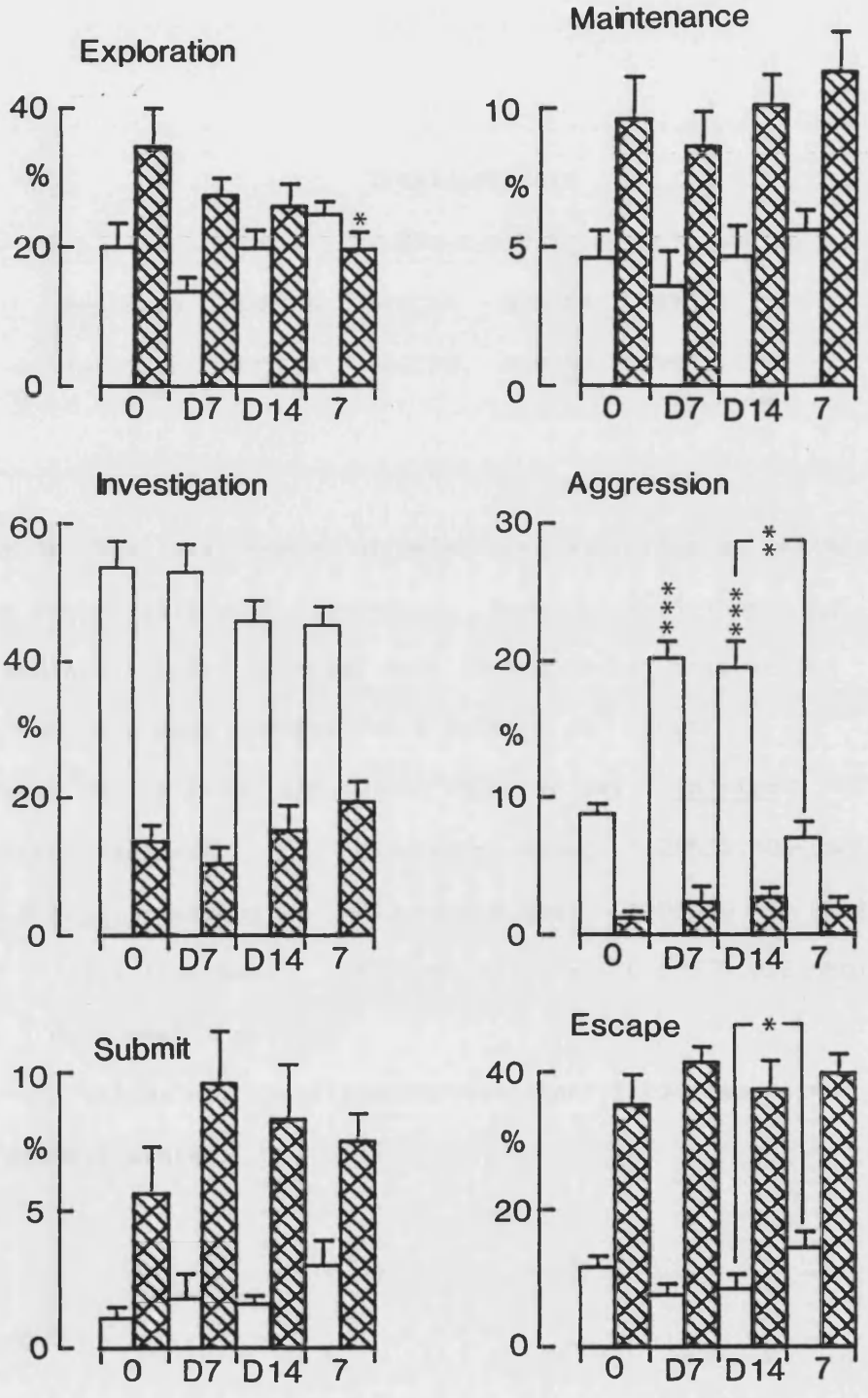
0 : Pre-treatment Fluoxetine dose; 1.205±0.006 umol Kg^{-1}

D7 : 7 days treatment Fluoxetine dose; 1.085±0.010 umol Kg^{-1}

D14 : 14 days treatment Fluoxetine dose; 0.995±0.022 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to pre-treatment (day 0) except where indicated.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	Treatment days			
	0	D7	D14	7
Resident	485±48	614±41	576±54	497±36
Intruder	437±33	460±22	450±40	399±52

Table 5.18 The total number of behaviours exhibited by resident rats treated chronically with fluoxetine, target dose 1.1 umol Kg^{-1} day^{-1} sc., and non-treated intruder rats during social interaction.
Values indicate mean and sem for 8 animals per group.
Pump rate; 9.678 (n=4) and 10.584 (n=4) uL day^{-1} in vivo
0 : Pre-treatment Fluoxetine dose; 1.205±0.006 umol Kg^{-1}
D7 : 7 days treatment Fluoxetine dose; 1.085±0.010 umol Kg^{-1}
D14 : 14 days treatment Fluoxetine dose; 0.995±0.022 umol Kg^{-1}
7 : 7 days post-treatment
MWUT : All values not significantly different from respective pre-treatment score.

5.5.3.8 Haloperidol

The behavioural profiles exhibited by resident rats treated chronically with haloperidol, target dose 0.11 umol Kg^{-1} day^{-1} sc., and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig.5.18 and Table 5.19 respectively.

5.5.3.8.1 Resident animals

Resident rats treated chronically with haloperidol, target dose 0.11 umol Kg^{-1} day^{-1} sc., showed little or no change by day 7 of treatment in the levels of environmental exploration, maintenance behaviour, investigation of the conspecific intruder, flight-submit, flight escape behaviours or in the total number of behaviours observed during social interaction. By 14 days of drug treatment environmental exploration was significantly increased from 13.8±2.1% at pre-treatment to 20.8±2.0% ($p<0.05$). Resident rats demonstrated a progressive reduction in the level of aggression directed at the conspecific intruder rats from 8.5±0.8% at pre-treatment to 6.8±0.7% (not significant) and 5.6±0.5% ($p<0.05$) by days 7 and 14 of haloperidol treatment respectively. By 7 days after drug treatment the level of aggression had returned to the level observed prior to drug treatment, while environmental exploration was further increased to 26.5±5.1% ($p<0.05$ compared to pre-treatment level) concomitant with a significant decrease in flight-submit behaviour from 3.8±0.6% at 14 days of treatment to 1.2±0.5% ($p<0.01$). Compared to the values observed prior to haloperidol treatment the data suggest a slight, albeit not significant, reduction in both investigation of the conspecific intruder and flight-escape behaviour together with a slight increase

in maintenance behaviour. The total number of behaviours exhibited by resident rats were only significantly reduced at 7 days post-treatment compared to that observed prior to haloperidol treatment.

5.5.3.8.2 Intruder animals

During the period of haloperidol treatment of resident rats, non-treated intruder rats demonstrated increased investigation of the conspecific resident rats from 11.2±2.1% at day 0 to 19.8±3.0% and 22.1±3.5% ($p<0.05$ in both cases), at days 7 and 14 respectively, but no significant change in the levels of aggression nor flight-submit or flight-escape behaviour. By 7 days following haloperidol treatment of resident rats, intruder rats still exhibited elevated investigation of the conspecific resident rats, while there was a significant increase in flight-escape behaviour, a slight, albeit not significant, increase in flight-submit behaviour and a slight, not significant, reduction in aggressive behaviour. In addition, throughout the experiment there was a progressive and significant decrease in environmental exploration and increase in maintenance behaviour exhibited by intruder rats. The total number of behaviours exhibited by intruder rats were only significantly reduced at 7 days following haloperidol treatment of the resident rats.

Figure 5.18 Effect of chronic treatment with haloperidol, target dose 0.11 umol Kg^{-1} day^{-1} sc., on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction. Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 9.678 uL day^{-1} <u>in vivo</u>

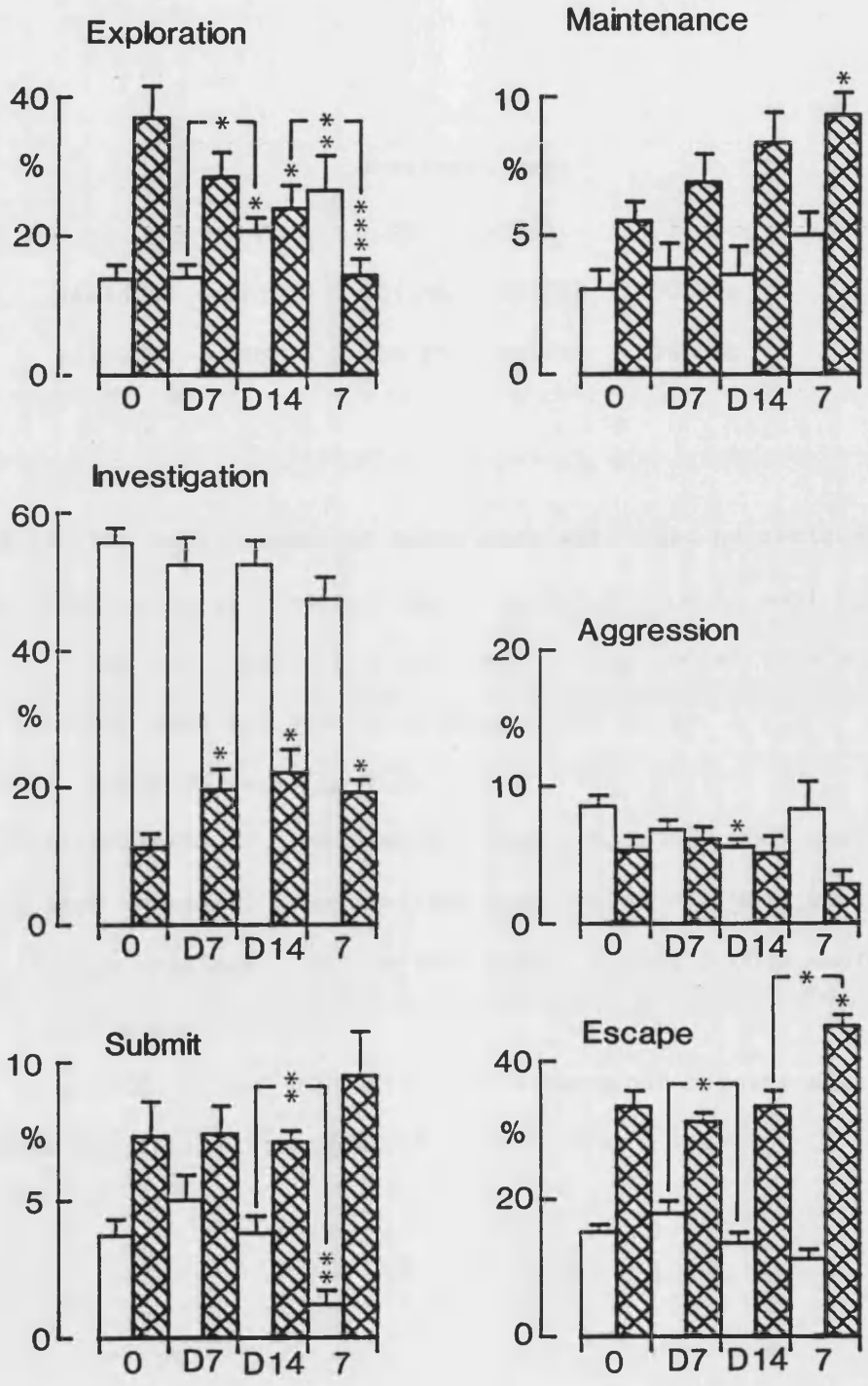
O : Pre-treatment Haloperidol dose; 0.1214±0.0007 umol Kg^{-1}

D7 : 7 days treatment Haloperidol dose; 0.1094±0.0011 umol Kg^{-1}

D14 : 14 days treatment Haloperidol dose; 0.0982±0.0018 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to pre-treatment (day O) except where indicated.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Escape
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D7
D14
7

	Treatment days			
	0	D7	D14	7
Resident	628±25	651±46	574±39	450±49a
Intruder	538±25	486±23	487±39	326±50b

Table 5.19 The total number of behaviours exhibited by resident rats treated chronically with haloperidol, target dose 0.11 umol Kg^{-1} day^{-1} sc., and non-treated intruder rats during social interaction. Values indicate mean and sem for 8 animals per group.

Pump rate; 9.678 uL day^{-1} in vivo

0 : Pre-treatment Haloperidol dose; 0.1214±0.0007 umol Kg^{-1}

D7 : 7 days treatment Haloperidol dose; 0.1094±0.0011 umol Kg^{-1}

D14 : 14 days treatment Haloperidol dose; 0.0982±0.0018 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : a, $p<0.05$; b, $p<0.01$. All other values not significant from respective pre-treatment score.

5.5.3.9 Diazepam-Vehicle

The behavioural profiles exhibited by resident rats treated chronically with diazepam-vehicle (see section 4.6 for details), and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.19 and Table 5.20, respectively. This experiment was designed as the control for chronic administration of diazepam.

5.5.3.9.1 Resident animals

Compared to the respective levels observed prior to the onset of treatment, resident rats treated chronically with diazepam-vehicle sc., showed no significant variation in environmental exploration, maintenance behaviour, investigation or aggression directed at the conspecific intruder or flight-escape behaviour either during the period of treatment or indeed at 7 days following the cessation of treatment. Only the level of flight-submit behaviour was observed to increase progressively from 1.4%±0.3% at pre-treatment to 3.1±0.8% (not significant) by day 14 of treatment, and this increase was maintained at day 7 post-treatment (2.8±0.5%, $p<0.05$ compared to the pre-treatment level). The total number of behaviours exhibited by resident rats during social interaction were slightly, but not significantly, higher at days 7 and 14 of treatment compared to the level observed prior to treatment.

5.5.3.9.2 Intruder animals

During the period of treatment of resident rats with diazepam-vehicle, non-treated intruder rats demonstrated increased investigation of the conspecific resident rats from 9.6±1.5% at day 0 to 13.6±1.6% (not significant) and 13.3±1.5% ($p<0.05$), at days 7

and 14 respectively. Indeed the elevated level of investigatory behaviour exhibited by intruder rats was still apparent at 7 days following the treatment of resident rats. The data also suggest a slight, albeit non significant, increase in aggressive behaviour directed at the resident rats and a slight, progressive, non-significant, increase in flight-submit behaviour which returned towards the level observed at day 0 by 7 days following the treatment the resident rats. Compared to the levels exhibited at day 0, intruder rats exhibited reduced levels in both environmental exploration and maintenance behaviour from day 7 of treatment and for the duration of the experiment, although these effects were not significant. In addition, the total number of behaviours exhibited by intruder rats were slightly increased from day 7 and for the duration of the experiment compared to the level observed at day 0.

Figure 5.19 Effect of chronic treatment with diazepam-vehicle (see section 4.6 for details) on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction. Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 10.584 uL day^{-1} in vivo

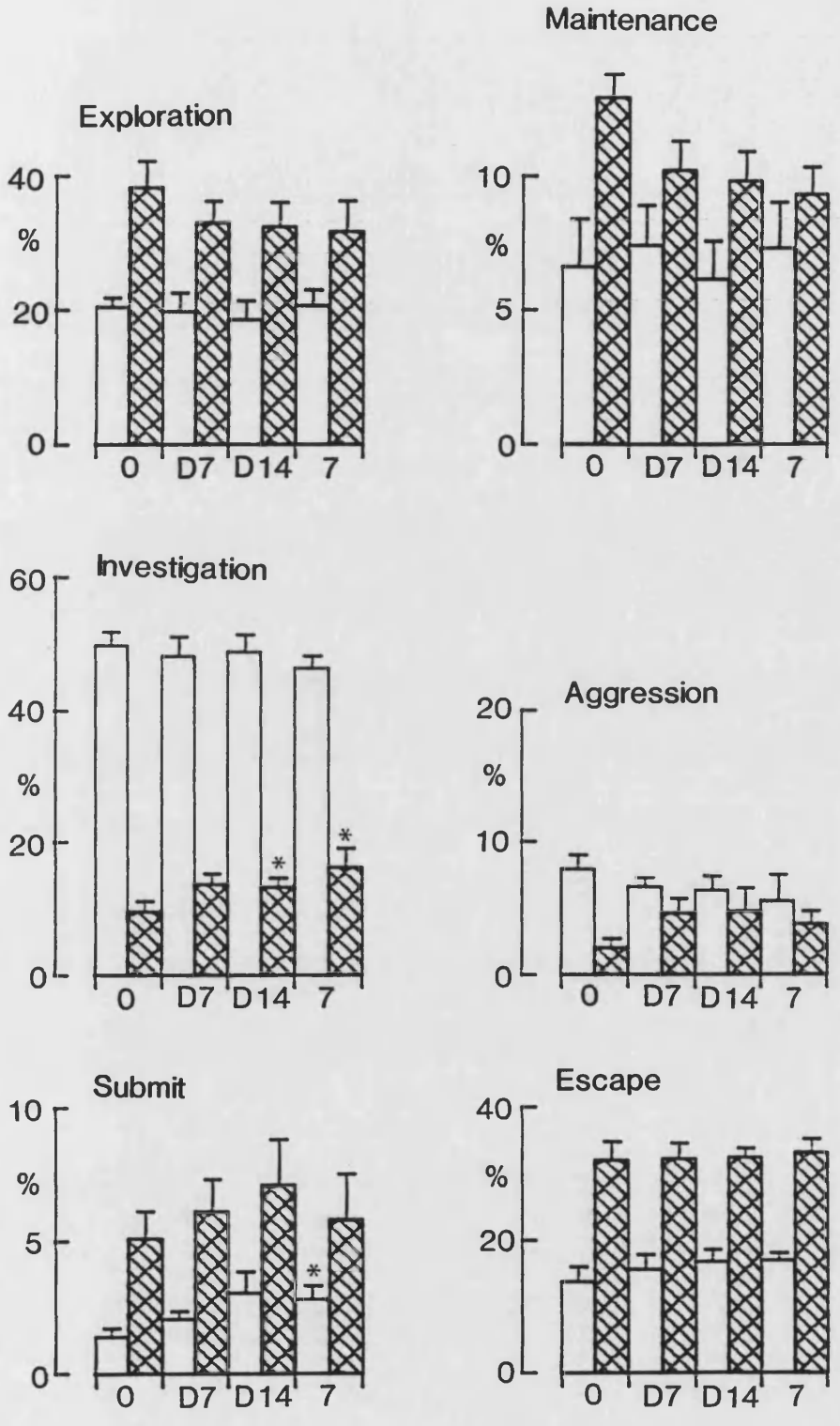
O : Pre-treatment

D7 : 7 days treatment

D14 : 14 days treatment

7 : 7 days post-treatment

MWUT : * $p<0.05$ compared to pre-treatment (day O).


Exploration
40
%
20
0
0
D7
D14
7
Maintenance
10
%
5
0
0
D7
D14
7
Investigation
60
40
%
20
0
*
*
0
D7
D14
7
Aggression
20
%
10
0
0
D7
D14
7
Submit
10
%
5
0
*
0
D7
D14
7
Escape
40
%
20
0
0
D7
D14
7

	Treatment days			
	0	D7	D14	7
Resident	485±57	589±49	588±54	543±40
Intruder	400±24	466±41a	462±42	472±38

Table 5.20 The total number of behaviours exhibited by resident rats treated chronically with diazepam-vehicle (see section 4.6 for details) and non-treated intruder rats during social interaction. Values indicate mean and sem for 8 animals per group.

Pump rate; 10.584 uL day^{-1} in vivo

0 : Pre-treatment

D7 : 7 days treatment

D14 : 14 days treatment

7 : 7 days post-treatment

MWUT : a, $p<0.05$. All other values not significant from respective pre-treatment score.

5.5.3.10 Diazepam

The behavioural profiles exhibited by resident rats treated chronically with diazepam, target dose 3.3 umol Kg^{-1} day^{-1} sc., and non-drugged intruder rats, and the total number of behaviours observed for each group are summarized in Fig. 5.20 and Table 5.21 respectively.

5.5.3.10.1 Resident animals

Resident rats treated chronically with diazepam, target dose 3.3 umol Kg^{-1} day^{-1} sc., exhibited increased flight-submit and flight-escape behaviours, from 1.7±0.5% and 14.4±1.7% at pre-treatment to 5.5±1.7% ($p<0.05$) and 23.8±1.7% ($p<0.01$) by day 7 of treatment respectively, concomitant with slight, albeit non-significant, reductions in the observed levels of investigation and aggression directed at the conspecific intruder rats. By 14 days of treatment both flight-submit and flight-escape behaviours were still significantly elevated compared to the respective levels observed prior to drug treatment. By 7 days following diazepam treatment the levels of flight-escape behaviour, investigation and aggression, but not flight-submit behaviour, had returned to those levels observed prior to drug treatment. No significant change was observed in the levels of environmental exploration or maintenance behaviour exhibited by resident rats either during, or indeed by 7 days following, drug treatment. Throughout the experiment, resident rats exhibited a progressive increase in the total number of behaviours observed during social interaction.

5.5.3.10.2 Intruder animals

By 7 days of chronic treatment of resident rats with diazepam, non-treated intruder rats exhibited increased investigation and aggression directed at the resident rats, from 11.8±1.3% and 2.8±0.7% at day 0 to 18.3±2.3% ($p<0.05$) and 7.8±2.2% (not significant) respectively, concomitant with a reduction in the level of environmental exploration, from 43.2±3.4% at day 0 to 32.6±3.5% ($p<0.05$), however, little or no significant change was observed in the levels of maintenance behaviour nor flight-submit or flight-escape behaviours. By day 14 of the experiment environmental exploration had decreased even further (to 25.2±3.8%, $p<0.01$ compared to the level at day 0), investigation and aggression were both still slightly, but significantly, elevated, while both flight-submit and flight-escape behaviours were increased compared to the respective levels observed at day 7, from 5.3±1.0% and 28.6±1.9% to 8.6±1.6% (not significant) and 35.8±1.8% ($p<0.05$) respectively. By 7 days following diazepam treatment of resident rats, intruder rats exhibited a further reduction in the level of environmental exploration, concomitant with further increases in the levels of flight-submit and flight-escape behaviours. Investigation of the conspecific resident rats was still significantly elevated at this time, however the elevated level of aggression, exhibited by intruder rats during the period of drug treatment of resident rats, had returned towards the level observed at day 0. No significant variation was observed in the level of maintenance behaviour nor in the total number of behaviours exhibited by intruder rats either during or following the period of diazepam treatment of resident rats compared to the levels observed at day 0.

Figure 5.20 Effect of chronic treatment with diazepam, target dose 3.3 umol Kg^{-1} day^{-1} sc., on the behavioural profile exhibited by resident and non-treated intruder rats during social interaction. Open columns, resident rats. Hatched columns, intruder rats. Columns indicate mean (and sem) percentage of total behaviours for each motivational category.

N=8 animals per group.

Pump rate; 9.678 uL day^{-1} in vivo

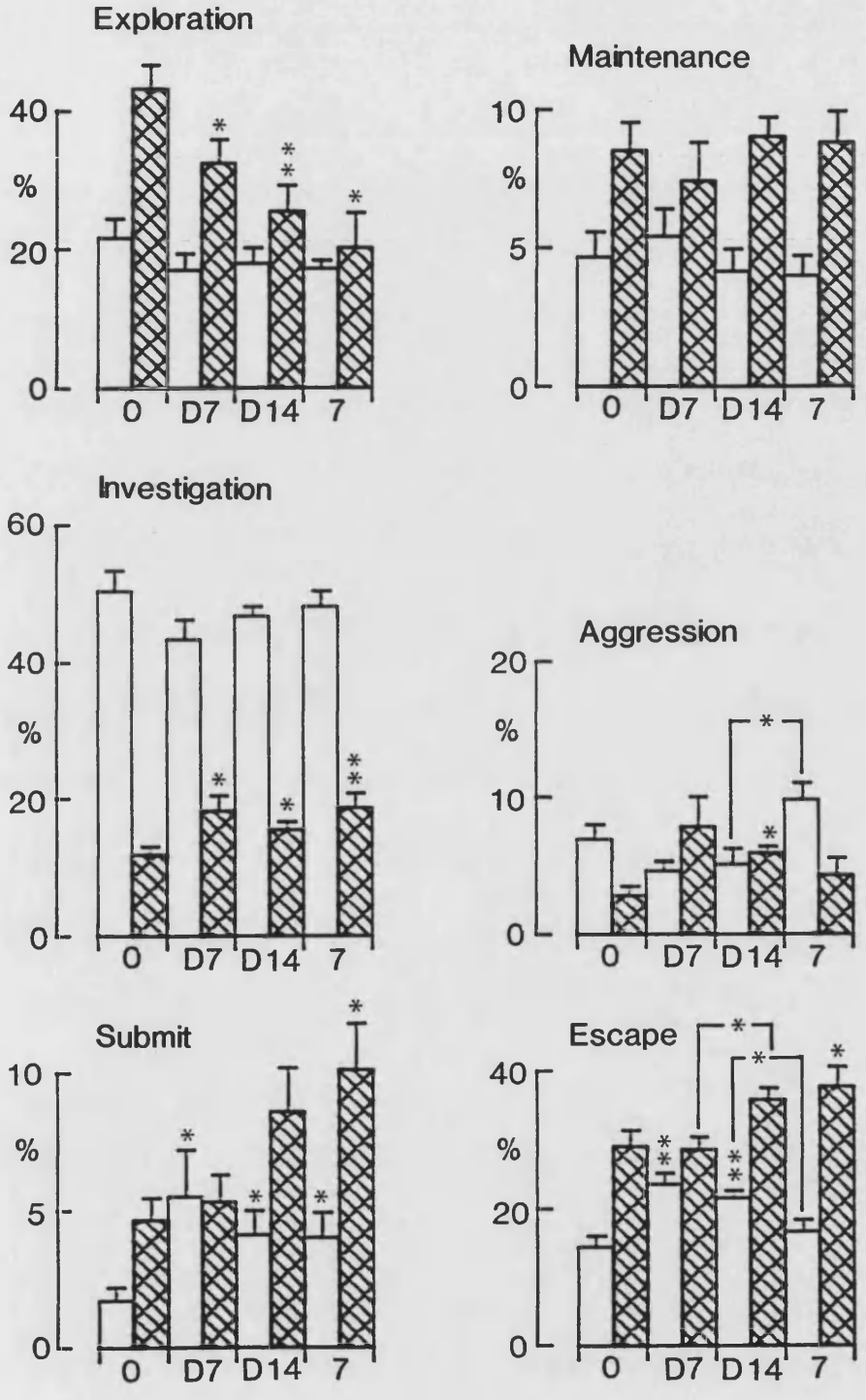
0 : Pre-treatment Diazepam dose; 3.598±0.014 umol Kg^{-1}

D7 : 7 days treatment Diazepam dose; 3.292±0.042 umol Kg^{-1}

D14 : 14 days treatment Diazepam dose; 3.026±0.050 umol Kg^{-1}

7 : 7 days post-treatment

MWUT : * $p<0.05$, ** $p<0.01$ compared to pre-treatment (day 0) except where indicated.


Exploration
40
%
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D7
D14
7
Maintenance
10
%
5
0
0
D7
D14
7
Investigation
60
40
%
20
0
0
D7
D14
7
Aggression
20
%
10
0
0
D7
D14
7
Submit
10
%
5
0
0
D7
D14
7
Escape
40
%
20
0
0
D7
D14
7

	Treatment days			
	0	D7	D14	7
Resident	599±24	659±52	710±33a	762±34b
Intruder	476±16	476±27	471±40	460±24

Table 5.21 The total number of behaviours exhibited by resident rats treated chronically with diazepam, target dose 3.3 umol Kg^{-1} day^{-1} sc., and non-treated intruder rats during social interaction.
Values indicate mean and sem for 8 animals per group.
Pump rate; 9.678 uL day^{-1} in vivo
0 : Pre-treatment Diazepam dose; 3.598±0.014 umol Kg^{-1}
D7 : 7 days treatment Diazepam dose; 3.292±0.042 umol Kg^{-1}
D14 : 14 days treatment Diazepam dose; 3.026±0.050 umol Kg^{-1}
7 : 7 days post-treatment
MWUT : a, $p<0.05$; b, $p<0.01$. All other values not significant from respective pre-treatment score.

5.6 Discussion

The major problem in observing animal behaviour and, more importantly, when using social behaviour as a pharmacological tool, is to ensure that the subject animals will perform not only at an adequate rate but also at the time required by the experimenter. Before any of the drug studies in this investigation were performed a number of decisions were made as to how to manipulate the environmental conditions to maximize the performance of the subjects. Thus social interaction experiments, between animals that were unfamiliar to each other, were performed following short-term isolation of the resident animal, during the dark phase of the light/dark cycle (although the area was under low illumination to enable video recording of the resulting social behaviour) when rodents are most active and in familiar conditions (i.e. the home cage of the resident animal). At all other times during the course of each experiment both resident and intruder animals were housed in social groups, since only then could the relative rank position of each animal be maintained (see section 5.2.1.1).

As many postures as possible were recorded during play-back of the video recording - since the limitations of such experiments are not the type or frequency of the behaviours exhibited but that which the observer can recognise and record - in order to approach as closely as possible a complete description of the profile of behaviour exhibited by each animal. It may be argued that the simple count of the total number of postures within any motivational category (used in these studies to represent the behavioural profiles) underestimates the importance of behavioural elements that last a relatively long period of time. For example, Aggressive Groom,

Crouch, Locomotion and the maintenance behaviours may last for many seconds, whereas Attack, Retreat, Threat/Thrust and Flag and Evade are usually of less than one second in duration. Also, the motivational categories contain varying numbers of behavioural elements such that it might be argued that this method of collating behavioural data is biased towards those categories containing a higher number of behavioural elements. In practice, however, the use of total values for each motivational category is not a serious problem since firstly, the longer the duration of a particular behavioural element, the less time is available for any other behaviour to be exhibited and thus the elements of long duration occupy a relatively greater proportion of the total number of behaviours exhibited; and secondly, where the motivational categories contain fewer behavioural elements, those elements are usually of longer duration and occur more frequently, e.g. locomotion and rearing. Other workers (e.g. File and Hyde, 1978) express data produced by similar experimental paradigms in terms of time spent in social interaction. Whether data expressed in the time domain yields more information is open to speculation since this method yields no indication of the type of behaviours expressed either during social interaction or when the animals are spatially separated. Expressing the data for each motivational category in terms of the percentage of the total number of behaviours observed, rather than as the summation of the absolute number of occurrences of the behavioural elements within a motivational category, allows identification of the changes in the distribution of behaviour regardless of the total number of behaviours exhibited. The resulting levels of activity exhibited by both resident and intruder animals, invariably between 0.5 and 2 postures per second throughout

the 10 minute monitoring period, provided an adequate "window" of activity for each motivational category monitored, even where the pre-treatment level of a particular motivational category was low (i.e. <2% of the total number of behaviours exhibited). The environmental conditions in which these investigations were performed were therefore adequate to meet the aims of the investigation.

In comparing sets of multi-distributed data it is usual to subject the data to X^2 analysis, since this statistical technique does not rely on the data being normally distributed and makes little assumption about the magnitude of the data collected. While being perfectly valid for the comparison between the behavioural profiles of resident and intruder animals exhibited during social interaction, the validity is lost when applied to the analysis of drug effects on one group of animals since it ignores the variation in response between individual animals to a particular dose of the drug being studied. In addition X^2 treats the motivational categories as independent and may therefore suggest several effects where there is really no more than one. Thus while such a test accurately locates the behaviour in which two groups of animals differ it nearly always over-estimates the significance of such differences. It is impossible to claim that the method used in these investigations identified and recorded every single behaviour or posture exhibited by the animals studied. Rather the data was gathered by sampling the behaviours exhibited during social interaction. A major criticism of this method is that it is subjective since the data collected is entirely dependent on the ability of the observer to identify correctly each behavioural element. It is therefore not possible to state that the data obtained by this method is normally distributed.

For this reason the non-parametric Mann-Whitney U-test was employed to determine significant drug effects on rodent social behaviour. The changes in the behavioural profiles exhibited by resident rats following either acute or chronic drug treatment, and indeed the indirect effects on the behaviour of intruder rats, were consistent between animals within each treatment group. Any conclusions arrived at by using the Mann-Whitney U-test are therefore believed to be broadly correct.

It is readily apparent that the behavioural profiles exhibited by resident and intruder animals are markedly different. Resident animals predominantly exhibit investigatory behaviour of the intruder conspecific, which occasionally progresses to aggressive acts directed at the intruder, while the intruder animals predominantly exhibit flight-escape behaviour rather than submissive postures in response to the investigation and aggression shown by the resident animals. This is indicative of the territorial advantage enjoyed by the resident animal brought about by short-term isolation together with the fact that social interaction occurs in the resident animals home cage. Resident animals are therefore inherently more dominant than the conspecific intruders.

The balance between the levels of flight-submit and flight-escape behaviour exhibited by intruder animals depends on the size of the area in which social interaction takes place (Grant, 1963); the smaller the area the lower the opportunity for the intruder animal to escape the attention of the resident animal. Intruder animals also show high levels of exploratory behaviour of the cage. The behaviour of intruder animals observed in a cage from which the resident animal

had been removed is primarily exploratory (88% of total behaviours). This indicates that environmental exploration is the primary objective of animals introduced into a potentially hazardous environment. Any reduction from this level of exploration observed during social interaction is indicative not only of the presence of the resident rats but also of the degree of attention received by the intruder.

The majority of grooming behaviours (i.e. licking, scratching and washing) observed in these studies for either animal invariably occurred immediately following social interaction and were both vigorous and of short duration. Resident animals demonstrated grooming behaviour when the opportunity to direct investigatory behaviour or aggression at the conspecific was frustrated (either by the conspecific showing flight-escape behaviour or becoming aggressive towards the resident rat). Intruder rats exhibited grooming behaviour when the opportunity to evade the investigation or aggression of the resident rats was thwarted. In both instances the grooming behaviour is indicative of Displacement Activity (Grant and Mackintosh, 1963; Silverman, 1965) implying motivational conflict. Displacement activities occur when an animal either desires to perform a certain behaviour but is frustrated from doing so and thus displays an alternative behaviour which appears quite irrelevant, or where two or more incompatible drives are strongly activated (e.g. attack and flight) such that each drive prevents the expression of the other (Bastock et al., 1953).

The effects of acute drug treatment of resident rats on the behavioural profiles exhibited by resident and non-treated intruder

rats are summarized in Table 5.22. Acute treatment of the resident rats with any of the drugs tested predominantly reduced the level of aggression directed at the intruder rats concomitant with increased flight-escape rather than flight-submit behaviour.

Figure 5.21 compares the dose-response curves of the compounds on aggressive behaviour exhibited by resident rats; the resulting order of potency being haloperidol >> mianserin > fluoxetine = iprindole > phenelzine > diazepam > clomipramine. When considered as a group the antidepressants induced no other consistent effects on the behavioural profile of resident rats. However, acute treatment with the antipsychotic haloperidol or the anxiolytic diazepam both reduced the total number of behaviours exhibited by the resident rats and increased the proportion of maintenance behaviours concomitant with the aforementioned reduction in aggressive behaviour. In addition, haloperidol concomitantly reduced flight-submit behaviour, while diazepam concomitantly reduced the level of environmental exploration and slightly reduced the level of investigatory behaviour directed at the conspecific intruder.

With the exception of diazepam, the drug-induced reduction in aggressive behaviour was not associated with reduced investigation of the conspecific intruder. This suggests that the antidepressants and haloperidol reduce the probability of the resident animals behaviour progressing from investigation of the conspecific to aggression, while for diazepam the reduced aggression was due, at least in part, to the reduced level of investigatory behaviour.

Resident Rats (treated)

	Motivational Category						
	E	M	I	A	FS	FE	T
	abc	abc	abc	abc	abc	abc	abc
Clomipramine	+			---	+	++	-
Fluoxetine			-	--	+	+++	
Iprindole				--		++	
Mianserin				--		++	
Phenelzine			-	---		++	
Haloperidol		++		--	--	++	---
Diazepam	--	++	--	--		+++	---

Intruder Rats (non-treated)

	Motivational Category						
	E	M	I	A	FS	FE	T
	abc	abc	abc	abc	abc	abc	abc
Clomipramine	+				--	--	
Fluoxetine							
Iprindole							-
Mianserin							
Phenelzine						-	
Haloperidol	++	+++		---	---	--	-
Diazepam	++				--	--	--

Table 5.22 Summary profile of drug-induced effects following acute treatment on rodent social behaviour exhibited by treated resident rats and non-treated intruder conspecifics.

Motivational Categories : E, Exploration; M, Maintenance;
I, Investigation; A, Aggression;
FS, Flight-submit; FE, Flight-escape;
T, Total number of behaviours.

a, b, c : Ascending doses of drug (umol Kg^{-1} sc);
Clomipramine, 10, 30, 90;
Fluoxetine, 1.1, 3.3, 10;
Iprindole, 1, 3, 9;
Mianserin, 0.33, 1, 3;
Phenelzine, 1, 3, 9;
Haloperidol, 0.11, 0.33, 1;
Diazepam, 3.3, 10, 30

\+ : Significant increase ($p<0.05$)

\- : Significant decrease ($p<0.05$)

Blank : No significant change

Figure 5.21 Effect of acute treatment with psychotropic drugs on aggressive behaviour exhibited by resident rats during social interaction.

Each point represents mean (and sem) percentage reduction from aggression exhibited by resident rats treated with drug-vehicle.

MWUT : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to respective vehicle control.

	ID_{50} (umol Kg^{-1}) (95% limits)
▲ Clomipramine,	29.5 (14.3,60.0)
■ Fluoxetine,	3#
♦ Iprindole,	3.82 (2.50,6.25)
● Mianserin,	1.19 (0.16,17.4)
▼ Phenelzine,	5.69 (1.41,221.5)
△ Haloperidol,	0.19 (0.09,0.33)
○ Diazepam,	8.73 (6.03,12.3)

ID_{50} values were calculated by use of the least-squares method.

The ID_{50} value for fluoxetine was estimated from the dose-response curve.

Mean % change (sem) in proportional aggression.
-100
-75
-50
-25
0
25
0.1
1.0
10.0
100.0
µmol/Kg sc

Non-treated intruder rats demonstrated little or no consistent change in their behavioural profile when in social interaction with resident rats treated acutely with any of the antidepressants. When in social interaction with resident rats treated with either haloperidol or diazepam, however, intruder rats demonstrated markedly reduced levels of both flight-submit and flight-escape behaviours and marked elevations in the level of environmental exploration. The reduced levels of social interaction instigated by the resident rats thus allows the intruder conspecific to exhibit its primary motivational target of environmental exploration rather than exhibiting aggressive behaviour directed at the conspecific resident. Aggressive behaviour exhibited by intruder rats was usually limited in response to investigation or aggression exhibited by the resident animal and is thus indicative of defensive behaviour (Blanchard and Blanchard, 1977; Blanchard et al., 1977). In addition, both groups of intruder rats demonstrated a reduction in the total number of behaviours in response to that observed in the corresponding resident group of rats. These observations indicate that haloperidol and diazepam both have far broader effects on rodent social behaviour than the antidepressants at doses that are equi-potent on aggressive behaviour.

The ID_{50} values for haloperidol or diazepam on aggressive behaviour exhibited by resident rats are nearly identical to those determined for the drug-induced reduction in the total number of behaviours (see sections 5.5.2.6.1 and 5.5.2.7.1 respectively). This would suggest that the reduction in aggressive behaviour induced by haloperidol or diazepam is due to the onset of overt sedation; the indirect effect of which is to allow the non-treated intruder rats to exhibit

increased environmental exploration. This being so the antidepressant-induced reduction in aggressive behaviour would appear to be a specific effect. This suggestion, however, is somewhat tentative. Aggressive behaviour occurs towards the final stages of the behavioural pathway (see Fig. 5.22), and may therefore be more sensitive to the sedative effects of psychotropic compounds. Drug-induced reduction in the level of aggressive behaviour may therefore be simply a more sensitive measure of the onset of drug-induced overt sedation. Exploratory locomotor activity in rodents has been suggested to be a sensitive measure of the possible sedative effects of psychotropic drugs (Brown et al., 1985). Experiments were therefore performed using this animal model to examine whether the drug-induced reduction in aggressive behaviour may be attributable to the onset of overt sedation and are described in chapter 6.

In summary, acute treatment of resident rats with the antidepressants resulted primarily in a reduction in the intensity of social interaction which, it is suggested, may be indicative of decreased social drive. Acute treatment with haloperidol, however, reduced both the intensity and level of social interaction while the effects observed following acute treatment with diazepam are attributable to reduced levels of social interaction only. The data suggest, albeit tentatively, that the antidepressant-induced reduction in aggressive behaviour may be a specific effect on rodent social behaviour and not due to the onset of overt sedation, while the effects on rodent behaviour following haloperidol or diazepam treatment may be due to motor impairment induced by the sedative effects of these two compounds.

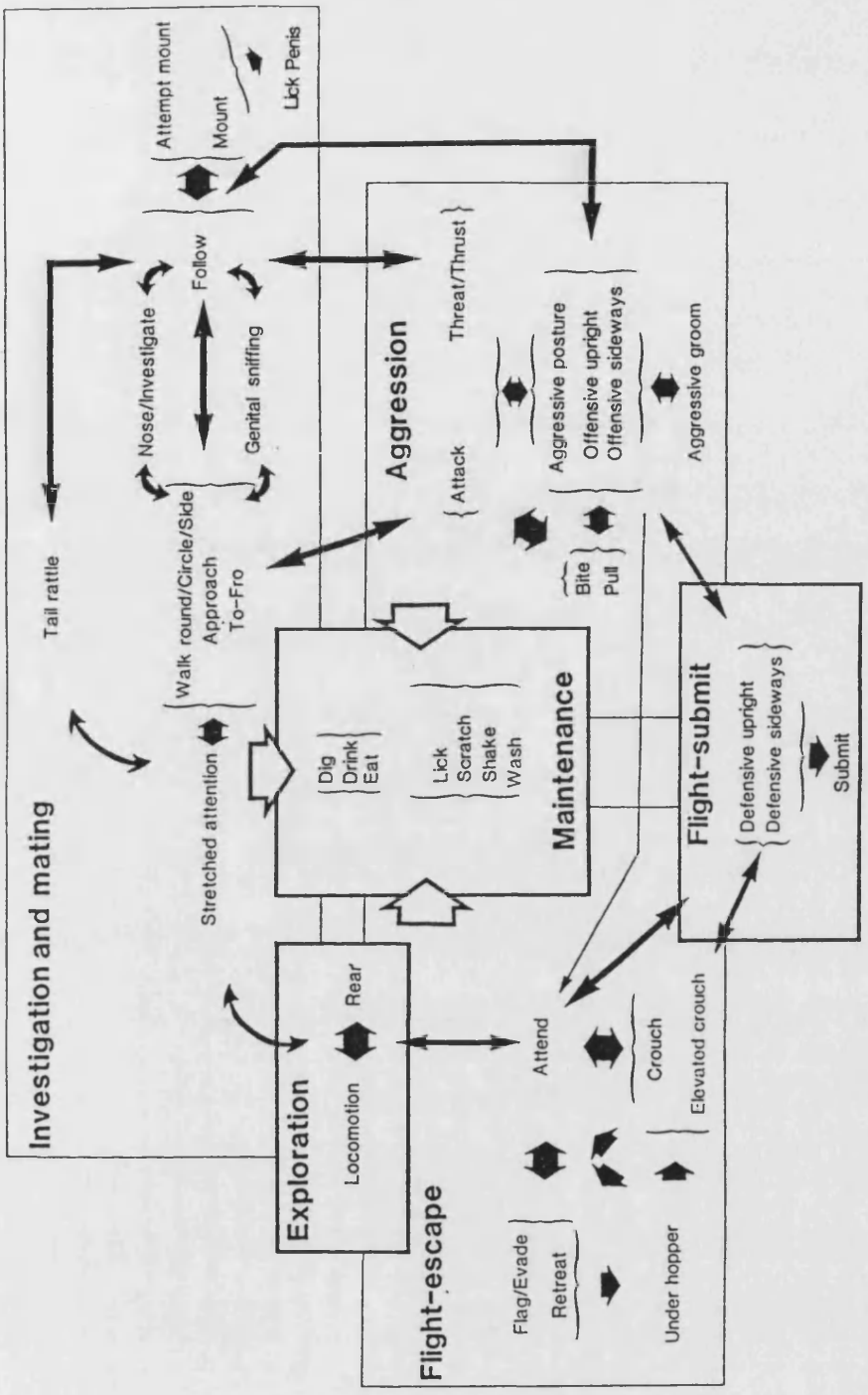


Figure 5.22 Simplified schematic representation of the pathways of social behaviour of the rat.

Individual postures and behaviours are grouped according to motivational category. Closed arrows indicate the normal progression of behaviours. Open arrows indicate displacement activity.

Adapted from Grant (1963).

The effects of chronic drug or drug-vehicle treatment of resident rats on the behavioural profiles exhibited by resident and non-treated intruder rats are summarized in Table 5.23. Resident rats treated chronically with the drug-vehicles H_2O or 0.1M tartaric acid exhibited increased flight-escape or flight-submit behaviour respectively. It is difficult to attribute such changes in the behavioural profile of the resident rats to the treatment schedules, rather the increased flight behaviours were probably due to the increased investigation exhibited by the intruder rats. Intruder rats also exhibited increased aggressive behaviour during social interaction with resident rats treated chronically with tartaric acid. Resident rats treated chronically with diazepam-vehicle, however, showed little or no variation in their behavioural profile during treatment even though the respective non-treated intruder rats exhibited increased investigation. These observations suggest that as a result of the repeat testing regimes intruder rats, which prior to day 0 are unaccustomed to the experimental method, become increasingly conditioned to social interaction with unknown resident animals in the latters home cage since it is known that previous social experience may condition the subsequent social behaviour in an agonistic situation (Baenninger, 1974; Bolhuis et al., 1984). It is suggested that such conditioning of intruder animals results in a reduction in the degree of territorial advantage enjoyed by the resident rats.

Chronic treatment of the resident rats with the antidepressants predominantly increased the level of aggression directed at the intruder conspecifics without concomitant increases in the level of investigation. As a direct result of the increased aggressive

Resident Rats (treated)

	Motivational Category						
	E	M	I	A	FS	FE	T
	ab	ab	ab	ab	ab	ab	ab
H_2O						++	
Clomipramine (10)	-	-		++	+		+
Iprindole (3)				++			
Mianserin (0.33)				++	-	--	
Phenelzine (1)		--		++		-	+
0.1M Tartaric Acid			-		+	+	
Fluoxetine (1.1)				++			
Haloperidol (0.11)	+			-			
Diazepam Vehicle							
Diazepam (3.3)					++	++	+

Intruder Rats (non-treated)

	Motivational Category						
	E	M	I	A	FS	FE	T
	ab	ab	ab	ab	ab	ab	ab
H_2O		-		++			
Clomipramine		-	-		++		+
Iprindole		-			++		
Mianserin		--		+	++	+	
Phenelzine		-			++	+	
0.1M Tartaric Acid	-			+	+		
Fluoxetine							
Haloperidol		-		++			
Diazepam Vehicle				+			+
Diazepam		--		++	+		

Table 5.23 Summary profile of drug-induced effects following chronic treatment on rodent social behaviour exhibited by resident rats and non-treated intruder conspecifics.

Values in parentheses indicate target daily doses of drug (umol Kg^{-1} sc)

Motivational Categories : E, Exploration; M, Maintenance;
I, Investigation; A, Aggression;
FS, Flight-submit; FE, Flight-escape;
T, Total number of behaviours.

a : Day 7 of drug treatment

b : Day 14 of drug treatment

\+ : Significant increase ($p<0.05$)

\- : Significant decrease ($p<0.05$)

Blank : No significant change

behaviour of the resident rats the non-treated intruder animals (with the exception of those in social interaction with resident rats treated with fluoxetine) exhibited increased flight-submit rather than flight-escape behaviour concomitant with decreased environmental exploration. Conversely neither chronic treatment with haloperidol nor diazepam increased the aggressive behaviour of resident rats, indeed such behaviour was reduced during haloperidol treatment. With the exception of resident rats treated chronically with the MAOI phenelzine the elevated levels of aggression returned to near pre-treatment levels by 7 days after drug treatment. The elevated level of aggression exhibited by resident rats treated chronically with phenelzine, however, only returned to the pre-treatment level at 14 days post-dose. These observations indicate that clomipramine, fluoxetine, iprindole and mianserin induce similar overall changes in the social behaviour of rats that are entirely dependent on the continued presence of the drug. Phenelzine, however, is an irreversible inhibitor of MAO such that full MAO activity may only be regained following the synthesis of new enzyme, which in the rat takes about 14 days. The effects of chronic phenelzine treatment on rodent behaviour therefore appear to have a direct biochemical correlate. The fact that the elevated levels of aggression returned to near-baseline levels post-treatment is surprising since aggressive behaviour is self-reinforcing (Baenninger, 1974; Taylor, 1979) and thus the level of aggressive behaviour exhibited post-treatment would be expected to be higher than that observed prior to drug treatment.

Resident rats treated chronically with haloperidol demonstrated elevated levels of environmental exploration which were further increased 7 days following the cessation of drug treatment such that

it is difficult to attribute this change in behaviour to drug treatment. Conversely the reduced level of aggressive behaviour observed at day 14 of drug treatment returned to the pre-treatment level by 7 days following the cessation of treatment concomitant with a comparatively large reduction in flight-submit behaviour. Chronic haloperidol treatment therefore appears to reduce the intensity of social interaction which, unlike the effects induced by acute treatment, is not associated with concomitant decreased flight-submit behaviour or the total number of behaviours observed nor with concomitant increased flight-escape behaviour. This in turn allows the non-treated intruder rats to exhibit investigatory behaviour of the conspecific resident rats.

Chronic diazepam treatment of resident rats resulted in increased flight-submit and flight-escape behaviour, however only the latter returned to the pre-treatment level by 7 days post-treatment. Resident rats therefore appear more likely to exhibit flight behaviour following social interaction during chronic diazepam treatment even though the level of agonistic activity exhibited by such animals was not significantly modified. Since intruder animals in social interaction with diazepam-treated resident rats exhibited increased investigatory activity of the latter this suggests that, like the effect of chronic haloperidol treatment, similar treatment with diazepam also reduces the intensity, but not the level, of social interaction.

The elevated levels of aggression induced by chronic antidepressant treatment were not associated with increased levels of investigation nor consistent increases in the total number of behaviours observed.

This suggests that such treatment increases the probability of the resident animals behaviour progressing from investigation to aggression directed at the conspecific (see Fig. 5.22). Such changes in rodent behaviour are indicative of an increase in the intensity of social interaction brought about by increased social drive rather than an increase in the basal level of activity. The effects of chronic antidepressant treatment on the basal levels of rodent activity (as indicated by the effect of such drug treatment on exploratory locomotor activity in rats) and a more applicable animal model of the social drive of rats (as indicated by the hierarchical position of individual rats within a social group) are described in chapters 6 and 7 respectively.

In summary, chronic treatment of resident rats with the antidepressants, but not haloperidol or diazepam, primarily results in increased aggressive behaviour which, it is suggested, is a manifestation of increased social drive in this species. Furthermore, acute and chronic antidepressant treatments induce diametrically different effects on rodent social behaviour. It is known that chronic antidepressant treatments induce profound changes in the biochemistry of central neurotransmitters (e.g. 5-HT, NA and GABA; see section 1.5). It is therefore further suggested that the behavioural effects observed during chronic antidepressant treatment in these studies may result from long-term drug-induced changes in the function of central neurotransmitter systems which control rodent social behaviour.

The relationship of these results to the effects of acute and chronic treatment of antidepressants on exploratory locomotor activity and

social drive in rats together with their implications in relation to published investigations by other workers are discussed in detail in chapter 8.

CHAPTER 6 EXPLORATORY LOCOMOTOR ACTIVITY IN THE RAT

CHAPTER 6 EXPLORATORY LOCOMOTOR ACTIVITY IN THE RAT

6.1 Introduction

Many centrally-acting pharmacological agents (e.g. antidepressants, antipsychotics, anxiolytics, tranquilizers etc.) are sedative when given acutely and, although sedation may be required under certain circumstances, such an effect is usually classified under the heading of unwanted side-effects. Studies on the psychopharmacological manipulation of overt animal behaviour patterns must always account for the possible induction of sedation, which may be induced by a variety of pharmacological mechanisms, since many behavioural models are sensitive to the sedative effects of such compounds which may confuse the experimental results unless adequately controlled.

Drug-induced suppression of exploratory locomotor activity (ELA), where animals are introduced to a novel environment, or spontaneous locomotor activity, where animals have previously been habituated to the experimental environment, in rats or mice, have long been used to identify the potential sedative effects of psychotropic agents. ELA in mice has been demonstrated to be susceptible to inhibition by a range of compounds from different pharmacological classes, and in some cases the mechanism of action may be determined by the use of specific pharmacological antagonists; suppression of ELA induced by DA-autoreceptor agonists, alpha$_2$-NA agonists and the benzodiazepines, chlordiazepoxide and diazepam, may be reversed by spiperone (DA antagonist), idazoxan (alpha$_2$-NA antagonist) and Ro 15-1788 (benzodiazepine antagonist) respectively (Brown et al., 1985). Acute treatment with clomipramine, fluoxetine, iprindole, mianserin, phenelzine, haloperidol or diazepam demonstrably reduced

the level of aggression exhibited by resident rats in the social interaction test (section 5.5.2), which may be due to the onset of overt sedation, while chronic treatment with those compounds clinically labelled antidepressant, but not haloperidol or diazepam, increased the level of aggression (section 5.5.3). In order to determine whether or not the drug-induced reduction in aggressive behaviour of resident rats observed following acute treatment could be attributed to the onset of overt sedation, experiments were designed to identify the sedative potential of each compound on ELA of the rat. In addition, the level of ELA of the rat was monitored following chronic administration of each compound, at those doses employed in the social interaction experiments, to determine whether the increase in aggression observed following chronic treatment with the antidepressants was a consequence of increased basal activity of the rats.

6.2 Methods

6.2.1 Subjects

Male Wister rats were taken from stock on weaning and housed under reverse-daylight conditions (12h. on/12h. off, lights on 2000) for 5 weeks prior to each experiment, with standard laboratory chow (Labsure CRM diet) and water available ad libitum. All animals used in these studies were naive to the locomotor activity monitoring cabinet and only used on one occasion.

6.2.2 Basic Methodology

Locomotor activity experiments were carried out between 1100h and 1300h. Locomotor activity was measured in a microcomputer-controlled activity monitor containing 7 infrared photocell units (see section

4.4.2.3 for description) for the 10 min period immediately following introduction of the rat into the activity monitor. The total number of counts were recorded every minute.

6.2.2.1 Effect of acute drug treatment on ELA of the rat

All animals were housed in groups of 12 prior to the experiment. Rats were subcutaneously treated with test drug or vehicle either 30 min (clomipramine, fluoxetine, iprindole, mianserin, phenelzine or diazepam) or 60 min (haloperidol) prior to introduction to the activity monitor. Each experiment was performed over 2 consecutive days with the doses of each compound administered in random order.

6.2.2.2 Effect of chronic drug or drug-vehicle treatment on ELA of the rat

All animals were housed in groups of 6 prior to the experiment. 1 week prior to testing the rats were anaesthetised and an osmotic mini-pump, containing drug or drug-vehicle, implanted subcutaneously (see section 4.5 for implantation procedure). All animals within each group received identical drug (clomipramine, fluoxetine, iprindole, mianserin, phenelzine or haloperidol) or drug-vehicle, H_2O or 0.1M tartaric acid, treatment. On the day of testing, the exploratory locomotor activity of each animal was determined as described above. The effects of chronic diazepam or diazepam-vehicle on ELA in rats were not examined.

6.3 Statistical analysis

The data for each treatment group were collated and the mean number of counts and sem over the 10 min monitoring period calculated. Significant differences from respective controls were identified

using Dunnett's test for multiple comparisons. Regression data were analysed and ID_{50} values with 95% confidence limits determined by analysis of variance including correction for variance of respective controls.

6.4 Results

6.4.1 Effect of acute drug treatment on ELA of the rat

The effect of acute drug treatments on ELA of the rat are summarized in Table 6.1.

No significant effect on ELA of the rat was observed following acute subcutaneous treatment with clomipramine, 10-90 umol Kg^{-1}, fluoxetine, 1.1-10 umol Kg^{-1}, iprindole or phenelzine, 1-9 umol Kg^{-1} in both cases. Mianserin, 3, but not 0.33 or 1, umol Kg^{-1} sc, significantly increased ELA (+28±14%, $p<0.05$). Only acute subcutaneous treatment with either haloperidol or diazepam induced highly significant dose-related decreases in ELA (regression analysis of variance F ratios of 58.9 and 47.6 respectively, df=15,1 $p<0.001$ in both cases); ID_{50} values, 0.18 and 8.6 umol Kg^{-1} respectively.

6.4.2 Effect of chronic drug or drug-vehicle treatment on ELA of the rat

The effects of chronic drug treatment on ELA of the rat are summarized in Table 6.2.

None of the chronic drug treatments induced a significant change in the level of ELA compared to the level observed following chronic treatment with the respective vehicle control.

Drug	Dose (umol Kg^{-1})	Counts (Mean±sem)	% Change	ID_{50} (95% limits)	Regression F ratio
Clomipramine	H_2O	179±36			
	10	170±28	-12±13		
	30	154± 6	-20± 7		
	90	141±10	-24± 4	>90	2.6
Fluoxetine	Tartrate	202±10			
	1.1	221±31	+ 9±15		
	3.3	188±11	- 7± 6		
	10	188±13	- 7± 7	>10	1.3
Iprindole	H_2O	229±26			
	1	202±30	-12±13		
	3	224±28	- 2±12		
	9	249±39	+ 9±17	> 9	1.0
Mianserin	H_2O	197±26			
	0.33	202±22	+ 2±11		
	1	229±23	+16±11		
	3	252±28a	+28±14	> 3	2.2
Phenelzine	H_2O	157±25			
	1	178±24	+14±15		
	3	156±17	± 0±11		
	9	143±17	- 9±11	> 9	1.6
Haloperidol	Tartrate	245±12			
	0.11	153±14b	-38± 6		
	0.33	87±12b	-65± 5	0.18	58.9c
	1	26± 8b	-89± 3	(0.12,0.26)	
Diazepam	Vehicle	218±11			
	3.3	159±13b	-26± 6		
	10	93±13b	-57± 6	8.59	47.6c
	30	48± 8b	-78± 4	(5.75,12.5)	

Table 6.1 Effect of acute drug treatment on ELA of rats.

Values indicate mean and sem for 6 animals per group. % change; percentage change from vehicle control. Tartrate concentration = 0.1M; see section 4.6 for description of diazepam-vehicle.

Dunnett's test; a, $p<0.05$; b, $p<0.01$. All other values not significant from vehicle control

ID_{50} values with 95% confidence limits and regression data were determined by analysis of variance including correction for variance of controls. F ratio; c, $p<0.001$, where df=15,1

Drug	Pump rate ($uL\ day^{-1}$)	Dose ($umol\ Kg^{-1}\ day^{-1}$)	Counts (Mean±sem)
H_2O	26.88		208±23
Clomipramine		10	207±23
Iprindole		3	207±27
Mianserin		0.33	175±15
Phenelzine		1	197±26
0.1M Tartaric acid	26.88		188±26
Fluoxetine		1.1	180±12
Haloperidol		0.11	202±22

Table 6.2 Exploratory locomotor activity of rats following 7 days chronic subcutaneous treatment with drug or drug-vehicle.
Values indicate mean and sem for 6 animals per group.
Dunnett's test: All values not significant from respective vehicle control.

6.5 Discussion

Earlier experiments have demonstrated that acute treatment with clomipramine, fluoxetine, iprindole, mianserin, phenelzine, haloperidol or diazepam reduced the level of aggression exhibited by resident rats in the social interaction test (section 5.5.2), whereas, with the exception of haloperidol and diazepam, chronic treatment induced significantly increased aggressive behaviour (section 5.5.3). However, whether such drug-induced changes observed following acute treatment are indicative of the onset of overt sedation, or whether the increased aggression observed during chronic administration of the antidepressants is due to a drug-induced increase in basal rodent activity are not known.

It has been demonstrated that exploratory locomotor activity in mice, where the level of locomotion is stimulated by the novel environment, is a sensitive animal model to examine the ability of psychotropic drugs to induce overt sedation (Brown et al., 1985). Experiments were performed using this animal model in the rat to examine the sedative potential of acute treatment with these compounds at those doses employed in the social interaction test. Only acute treatment with haloperidol or diazepam induced dose-related inhibitions in the level of ELA indicating that both compounds induce overt sedation at those doses used. The dose-response curves obtained following acute treatment with these compounds on ELA are compared to those obtained on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction in figures 6.1 to 6.7. The data indicate that clomipramine (Fig. 6.1), fluoxetine (Fig. 6.2), iprindole (Fig. 6.3), mianserin (Fig. 6.4) and phenelzine (Fig. 6.5) were more potent on aggressive behaviour than on ELA or

the total number of behaviours exhibited during social interaction. Conversely, both haloperidol (Fig. 6.6) and diazepam (Fig. 6.7) exhibited equi-potent effects on all three parameters respectively. The data therefore indicate that the reduction in aggressive behaviour exhibited by resident rats following acute treatment with haloperidol or diazepam, but not the antidepressants, is due to the onset of overt sedation. In addition, acute treatment with any of the compounds exhibited very similar effects on ELA and the total number of behaviours observed during social interaction. This indicates that measurement of the total number of behaviours exhibited during social interaction yields the same information regarding the sedative potential of psychotropic drugs as the measurment of ELA. Measurement of the total number of behaviours exhibited by resident rats therefore provides an indication of any sedative effects of psychotropic compounds that may influence social behaviour and is inherent in the social interaction test. The reduction in aggressive behaviour exhibited by resident rats during social interaction following acute treatment with the antidepressants therefore appears to be a specific effect on rodent social behaviour.

Figure 6.1 Effect of acute treatment with clomipramine on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction, and exploratory locomotor activity in the rat.

Closed triangles, aggression; closed circles, total number of behaviours; closed squares, exploratory locomotor activity.

Values indicate mean (and sem) percentage change from behaviour exhibited by vehicle-treated control rats.

Significant changes in aggression (N=8) and total number of behaviours (N=8) were identified by MWUT (* $p<0.05$, *** $p<0.001$).

Exploratory locomotor activity (N=6); all values not significantly different from control (Dunnett's test).

	ID_{50} umol Kg^{-1} (95% limits)
Aggression	29.5 (14.3,60.0)
Total number of behaviours	>90
Exploratory locomotor activity	>90

Aggression ID_{50} value was calculated by the least-squares method.

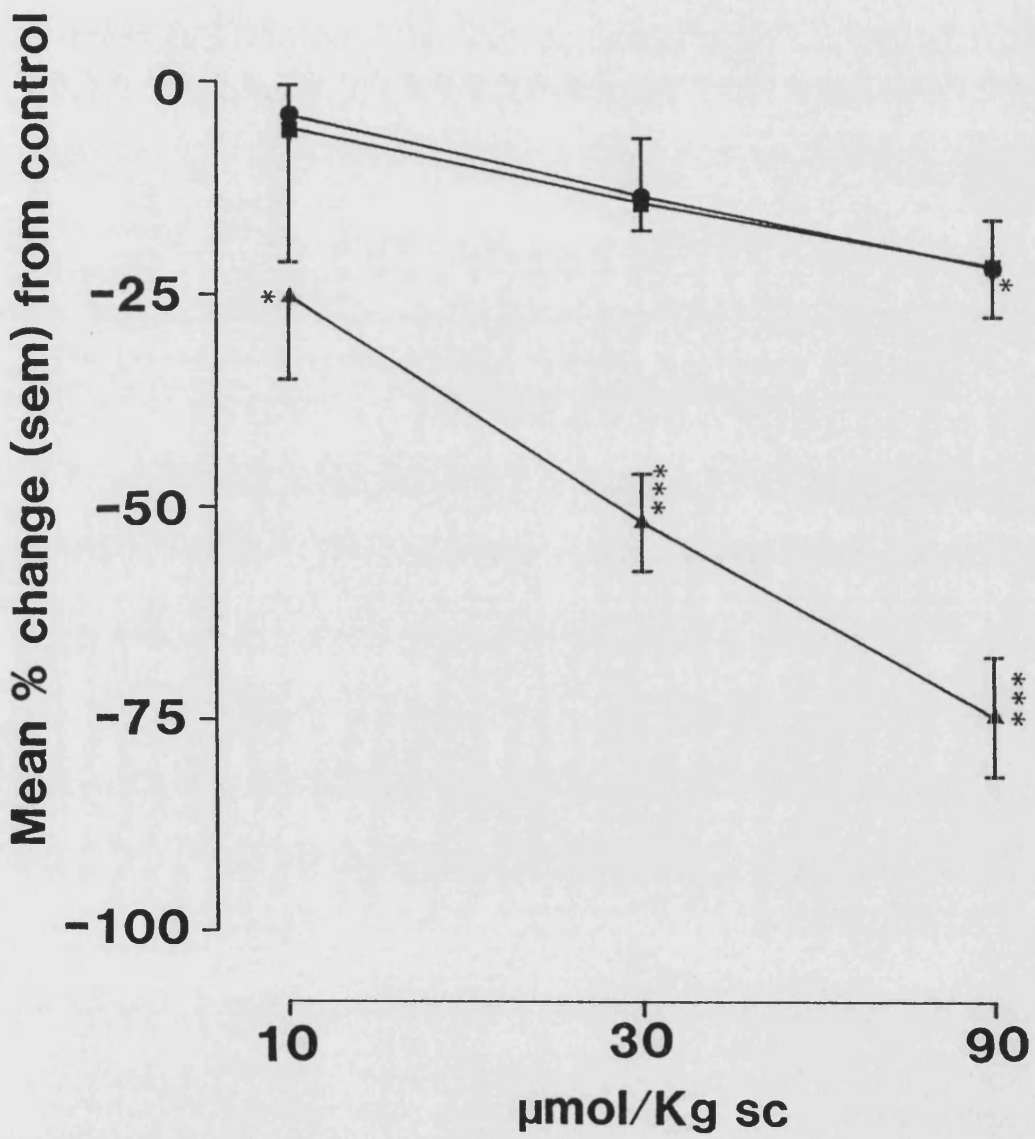

Mean % change (sem) from control
0
-25
-50
-75
-100
10
30
90
µmol/Kg sc

Figure 6.2 Effect of acute treatment with fluoxetine on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction, and exploratory locomotor activity in the rat.

Closed triangles, aggression; closed circles, total number of behaviours; closed squares, exploratory locomotor activity.

Values indicate mean (and sem) percentage change from behaviour exhibited by vehicle-treated control rats.

Significant changes in aggression (N=8) and total number of behaviours (N=8) were identified by MWUT (* $p<0.05$).

Exploratory locomotor activity (N=6); all values not significantly different from control (Dunnett's test).

	ID_{50} umol Kg^{-1} (95% limits)
Aggression	3#
Total number of behaviours	>10
Exploratory locomotor activity	>10

Estimated from dose-response curve.

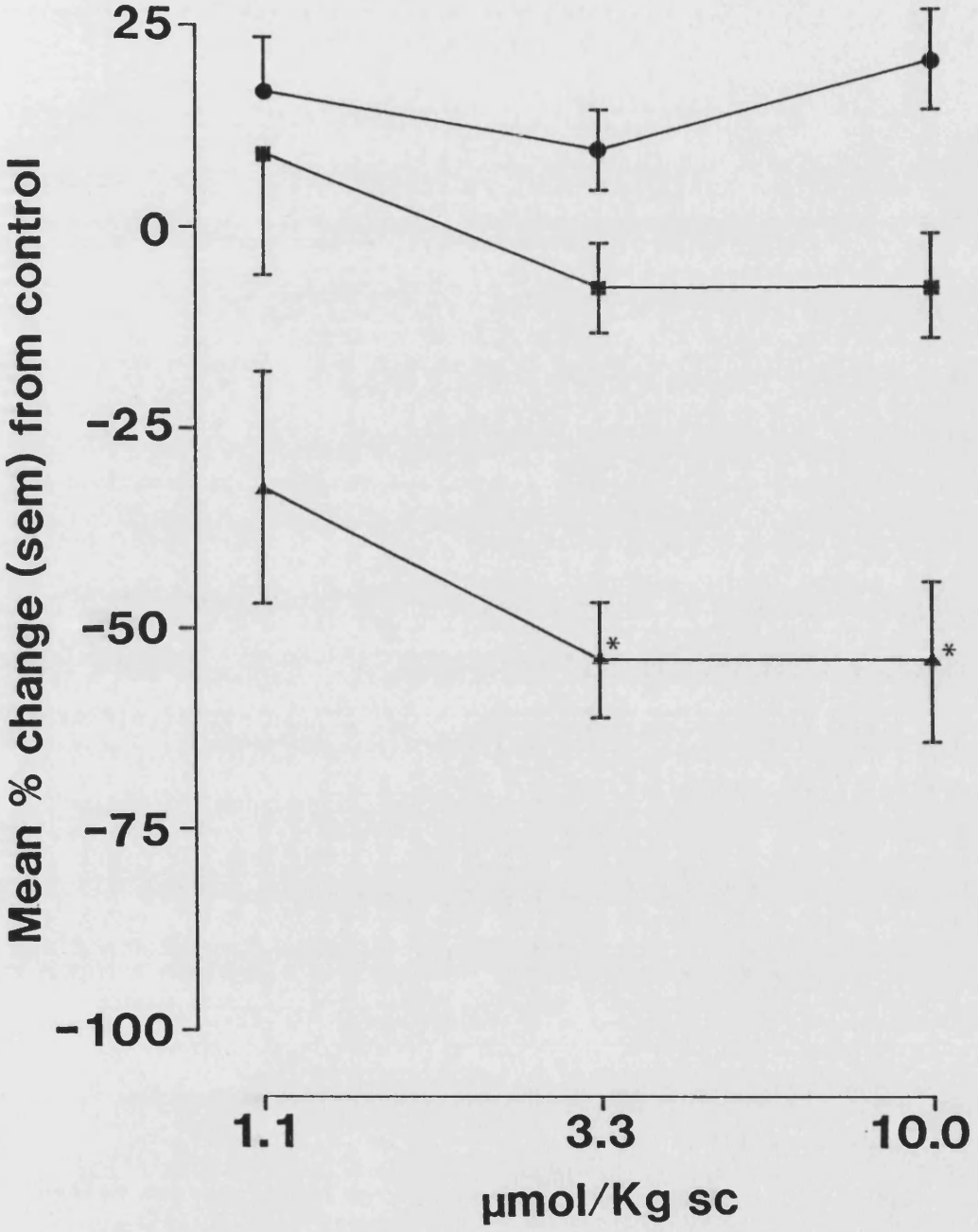

25
0
-25
-50
-75
-100
Mean % change (sem) from control
*
*
1.1
3.3
10.0
µmol/Kg sc

Figure 6.3 Effect of acute treatment with iprindole on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction, and exploratory locomotor activity in the rat.

Closed triangles, aggression; closed circles, total number of behaviours; closed squares, exploratory locomotor activity.

Values indicate mean (and sem) percentage change from behaviour exhibited by vehicle-treated control rats.

Significant changes in aggression (N=8) and total number of behaviours (N=8) were identified by MWUT (** $p<0.01$, *** $p<0.001$).

Exploratory locomotor activity (N=6); all values not significantly different from control (Dunnett's test).

	ID_{50} umol Kg^{-1} (95% limits)
Aggression	3.82 (2.50,6.25)
Total number of behaviours	>9.0
Exploratory locomotor activity	>9.0

Aggression ID_{50} value was calculated by the least-squares method.

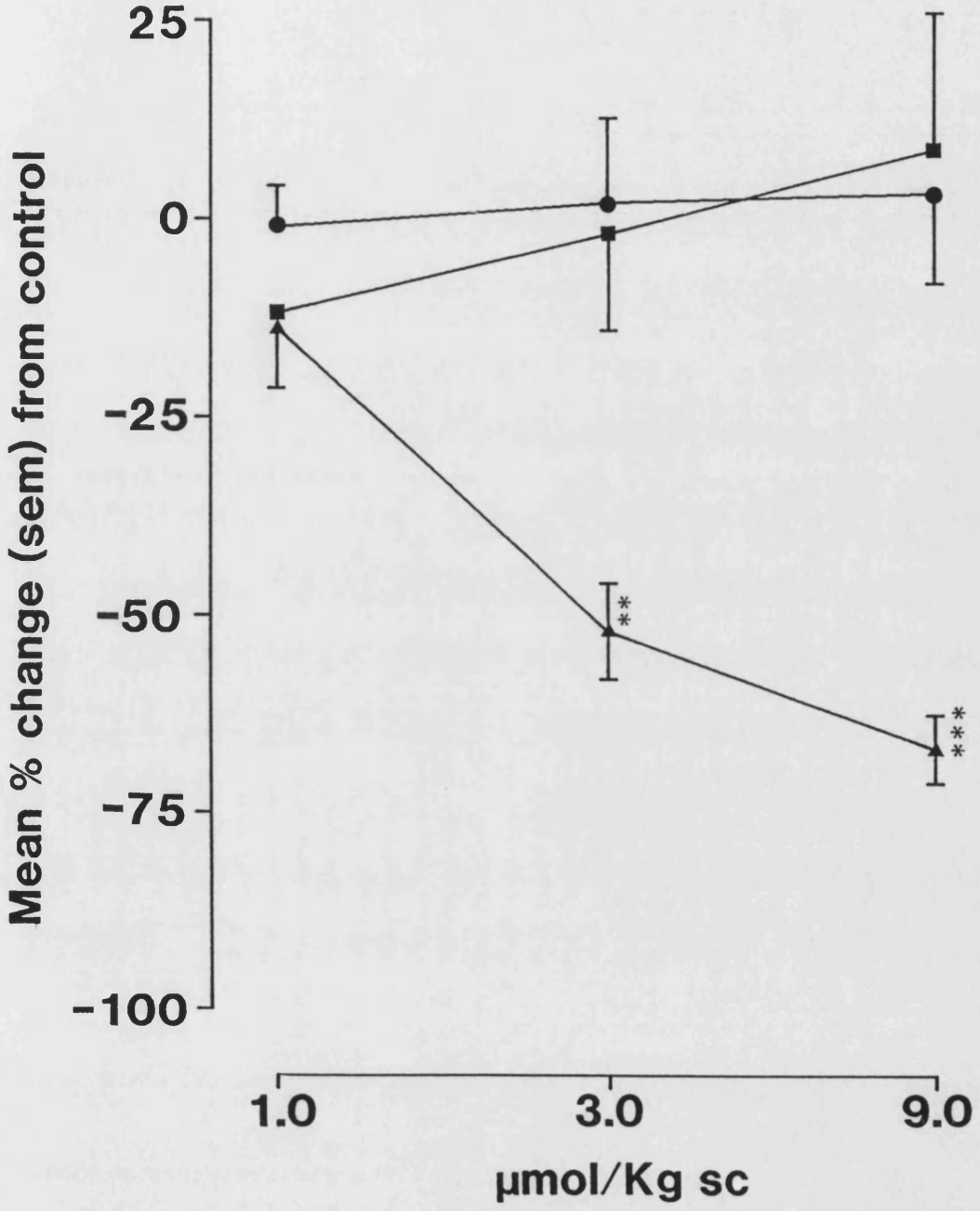

25
0
-25
-50
-75
-100
Mean % change (sem) from control
**
***
1.0
3.0
9.0
µmol/Kg sc

Figure 6.4 Effect of acute treatment with mianserin on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction, and exploratory locomotor activity in the rat.

Closed triangles, aggression; closed circles, total number of behaviours; closed squares, exploratory locomotor activity.

Values indicate mean (and sem) percentage change from behaviour exhibited by vehicle-treated control rats.

Significant changes in aggression (N=8) and total number of behaviours (N=8) were identified by MWUT (** $p<0.01$).

Significant changes in exploratory locomotor activity (N=6) were identified by Dunnett's test (* $p<0.05$).

	ID_{50} umol Kg^{-1} (95% limits)
Aggression	1.19 (0.16,17.4)
Total number of behaviours	>3
Exploratory locomotor activity	>3

Aggression ID_{50} value was calculated by the least-squares method.

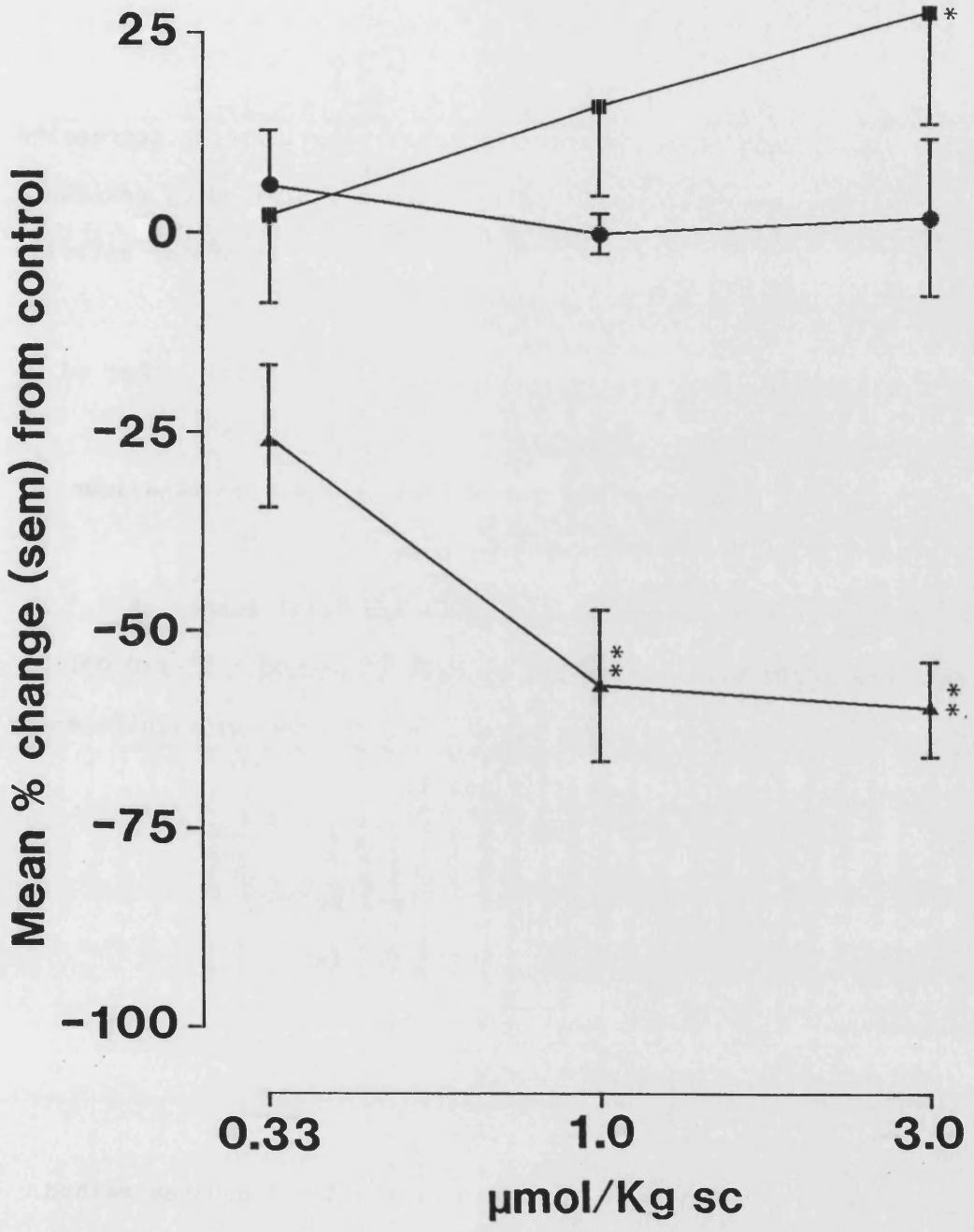

25
0
-25
-50
-75
-100
Mean % change (sem) from control
*
**
**
0.33
1.0
3.0
µmol/Kg sc

Figure 6.5 Effect of acute treatment with phenelzine on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction, and exploratory locomotor activity in the rat.

Closed triangles, aggression; closed circles, total number of behaviours; closed squares, exploratory locomotor activity.

Values indicate mean (and sem) percentage change from behaviour exhibited by vehicle-treated control rats.

Significant changes in aggression (N=8) and total number of behaviours (N=8) were identified by MWUT (* $p<0.05$, ** $p<0.01$).

Exploratory locomotor activity (N=6); all values not significantly different from control (Dunnett's test).

	ID_{50} umol Kg^{-1} (95% limits)
Aggression	5.69 (1.41,221.5)
Total number of behaviours	>9
Exploratory locomotor activity	>9

Aggression ID_{50} value was calculated by the least-squares method.

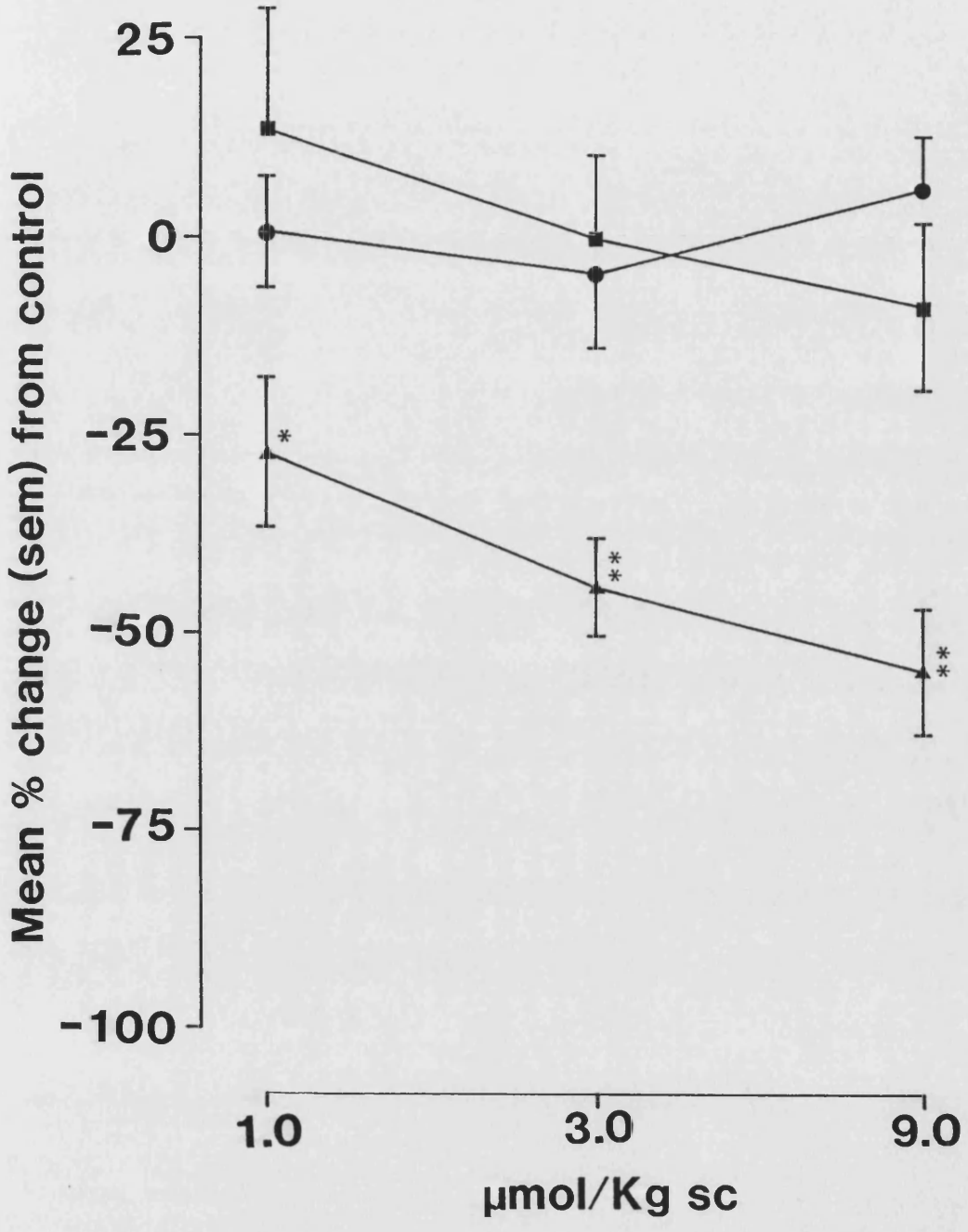

25
0
-25
-50
-75
-100
Mean % change (sem) from control
*
**
**
1.0
3.0
9.0
µmol/Kg sc

Figure 6.6 Effect of acute treatment with haloperidol on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction, and exploratory locomotor activity in the rat.

Closed triangles, aggression; closed circles, total number of behaviours; closed squares, exploratory locomotor activity.

Values indicate mean (and sem) percentage change from behaviour exhibited by vehicle-treated control rats.

Significant changes in aggression (N=8) and total number of behaviours (N=8) were identified by MWUT (** $p<0.01$, *** $p<0.001$).

Significant changes in exploratory locomotor activity (N=6) were identified by Dunnett's test (** $p<0.01$).

	ID_{50} umol Kg^{-1} (95% limits)
Aggression	0.19 (0.09,0.33)
Total number of behaviours	0.18 (0.09,0.29)
Exploratory locomotor activity	0.18 (0.12,0.26)

ID_{50} values for aggression and total number of behaviours were calculated by the least-squares method.

ID_{50} value for exploratory locomotor activity was calculated by analysis of variance including correction for variance of control.

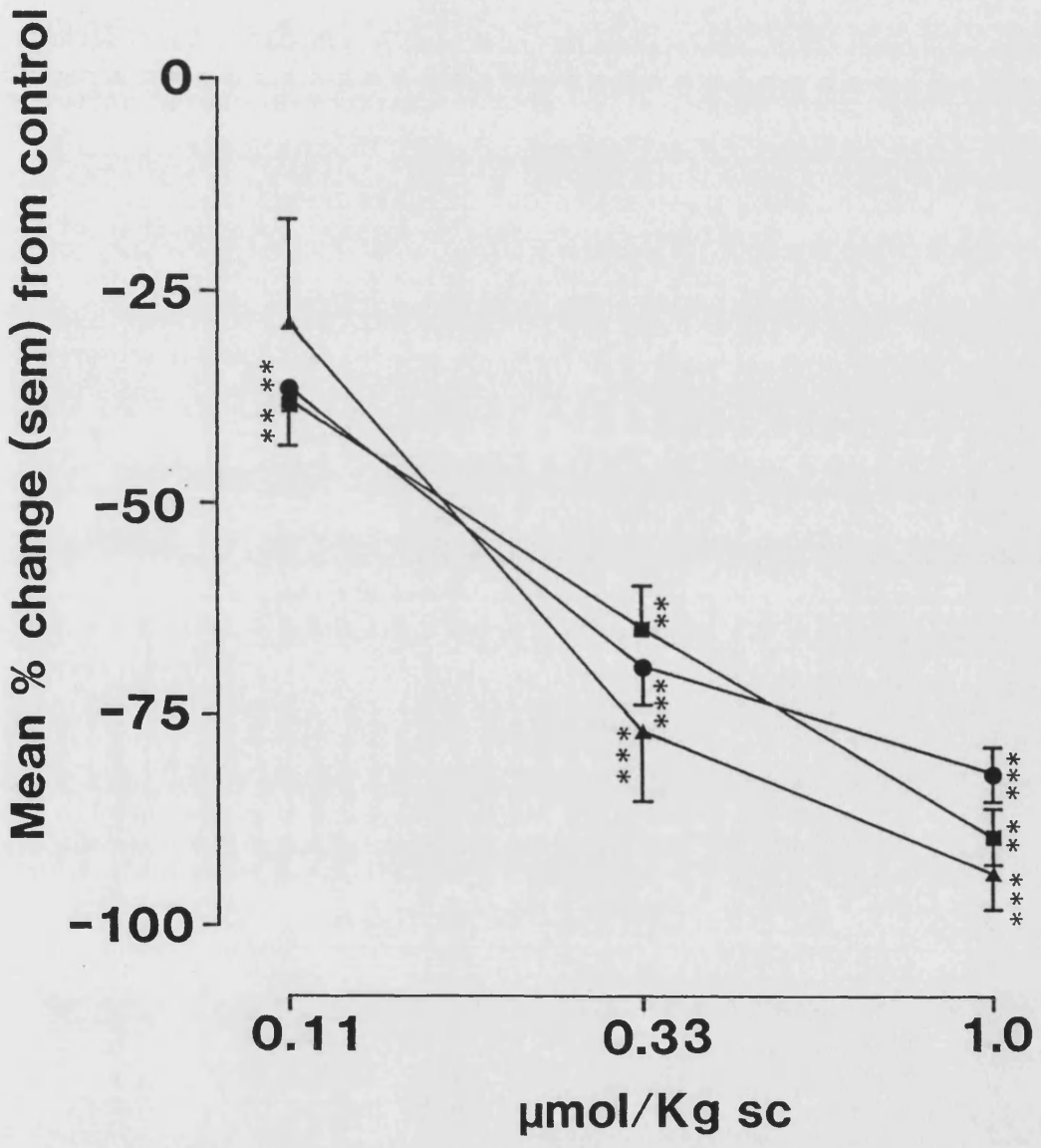

Mean % change (sem) from control
0
-25
-50
-75
-100
0.11
0.33
1.0
µmol/Kg sc

Figure 6.7 Effect of acute treatment with diazepam on aggressive behaviour and the total number of behaviours exhibited by resident rats during social interaction, and exploratory locomotor activity in the rat.

Closed triangles, aggression; closed circles, total number of behaviours; closed squares, exploratory locomotor activity.

Values indicate mean (and sem) percentage change from behaviour exhibited by vehicle-treated control rats.

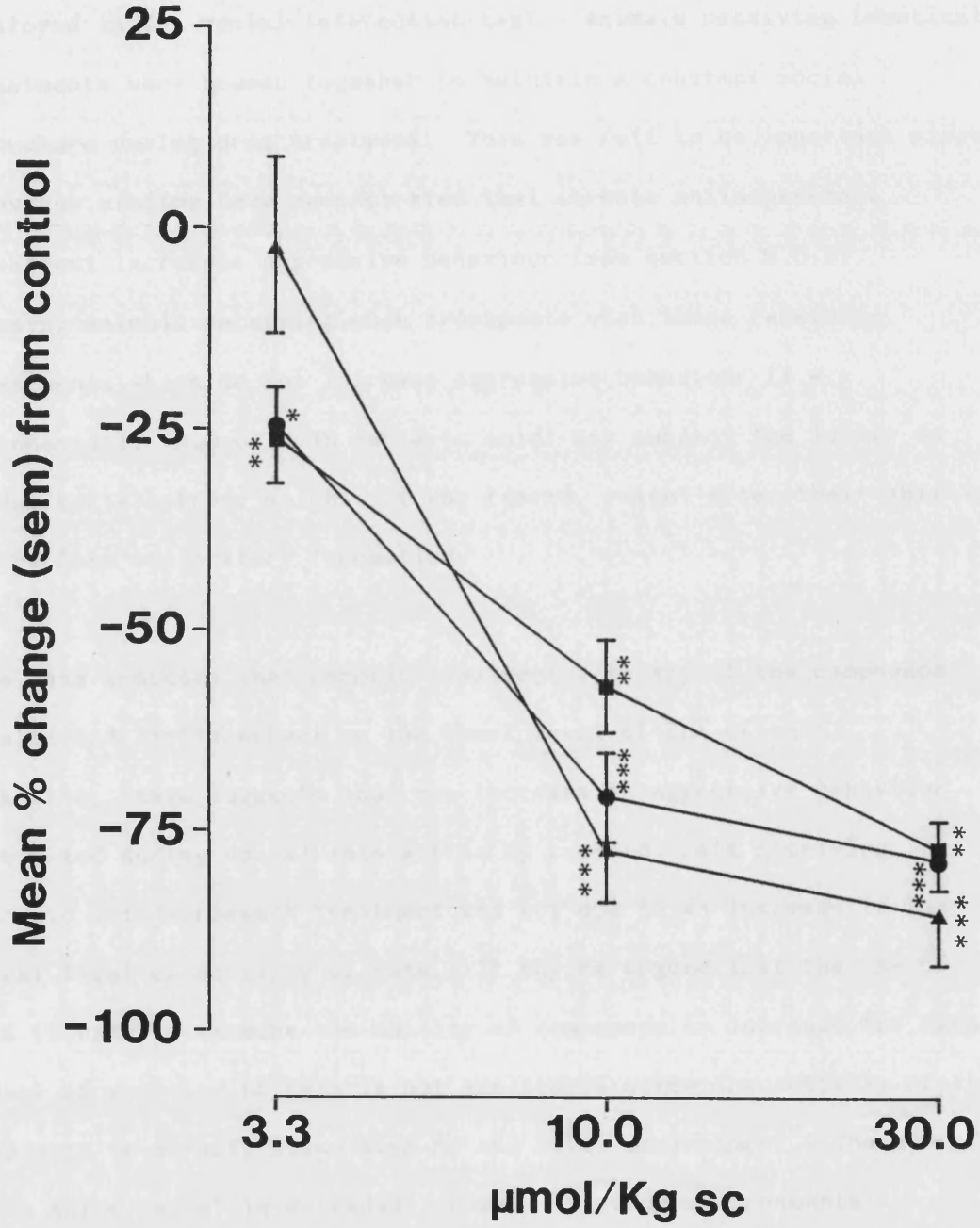
Significant changes in aggression (N=8) and total number of behaviours (N=8) were identified by MWUT (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Significant changes in exploratory locomotor activity (N=6) were identified by Dunnett's test (** $p<0.01$).

	ID_{50} umol Kg^{-1} (95% limits)
Aggression	8.73 (6.03,12.3)
Total number of behaviours	7.26 (5.05,9.96)
Exploratory locomotor activity	8.59 (5.75,12.5)

ID_{50} values for aggression and total number of behaviours were calculated by the least-squares method.

ID_{50} value for exploratory locomotor activity was calculated by analysis of variance including correction for variance of control.


25
0
-25
-50
-75
-100
Mean % change (sem) from control
3.3
10.0
30.0
µmol/Kg sc

In a separate series of experiments the effects of chronic treatment with clomipramine, fluoxetine, iprindole, mianserin, phenelzine or haloperidol on the ELA of rats were examined at those doses employed in the social interaction test. Animals receiving identical treatments were housed together to maintain a constant social structure during drug treatment. This was felt to be important since previous studies have demonstrated that chronic antidepressant treatment increases aggressive behaviour (see section 5.5.3). Housing animals receiving such treatments with those receiving treatments which do not increase aggressive behaviour (i.e. haloperidol, H_2O or 0.1M tartaric acid) may subject the latter to undue social stress which, it was feared, might alter their ability to perform exploratory locomotion.

The data indicate that chronic treatment with any of the compounds tested had little effect on the basal level of ELA of rats. Likewise, this suggests that the increase in aggressive behaviour exhibited during social interaction by resident rats receiving chronic antidepressant treatment was not due to an increase in the basal level of activity of rats. It may be argued that the use of ELA in rats to examine the ability of compounds to increase the basal level of activity of rats is not applicable since the activity of the subjects is already stimulated by the novel environment. The use of this animal model is defended, however, since environmental stimulation also occurs in the social interaction test by the presence of the intruder conspecific. ELA in rats, rather than the measurement of locomotor activity exhibited by rats habituated to the locomotor activity monitor, therefore mirrors more closely environmental stimulation inherent in the social interaction test.

Further experiments to examine the effects of chronic antidepressant treatment on the social drive of rats (as indicated by the hierarchical position of individual rats within a social group) are described in chapter 7.

In summary, acute treatment with those antidepressants studied in these investigations had little or no effect on the level ELA of rats. The reduced level of aggressive behaviour exhibited by resident rats in the social interaction test therefore appears to be due to a specific reduction in the intensity of social drive of rats, while that following acute treatment with haloperidol or diazepam appears to be as direct consequence of overt sedation. Conversely, since chronic treatment with these compounds had no effect on the level of ELA, the increased aggressive behaviour exhibited by resident rats receiving identical treatments during social interaction is probably due to a specific increase in the intensity of social drive as opposed to an increase in the basal level of activity.

The relationship of these results to the effects of acute and chronic treatment of antidepressants on the social behaviour of rats together with their implications in relation to published investigations by other workers are discussed in detail in chapter 8.

CHAPTER 7 RANK ORDER STUDIES IN THE RAT

rank order with a transitive dominance relationship, if animal A dominates animal B and B dominates C, then A dominates C. In non-linear rank orders at least one triadic dominance relationship must be intransitive such that if animal A dominates B and B dominates C, then C dominates A. Thus, while linear hierarchies are composed purely of transitive dominance relationships, non-linear hierarchies are composed of triads with both transitive and intransitive dominance relationships where the latter indicate a cyclical social structure.

An integral component of the establishment of rank order between male rats is the level of aggressive behaviour exhibited by each animal (Chance and Silverman, 1964). Thus the level of aggressive behaviour exhibited at grouping determines each animals' relative position within the social hierarchy, with higher aggressors attaining higher rank positions. Grant and Chance (1958) identified the rank order of caged laboratory rats by monitoring the full submissive posture exhibited by subordinate rats in response to the aggressive behaviour exhibited by a more dominant conspecific. Using this posture to identify not only the end point of a particular social interaction but also the dominant and subordinate rats within that interaction, they demonstrated that housing rats in groups of less than 6 animals per group allows for a stabilized linear rank order to be established for at least 6 weeks from 3 weeks following initial grouping. These observations imply that the level of aggression exhibited by each individual animal was relatively constant from one recording to the next. In addition Grant and Chance (1958) suggested that subordinate males are attracted to those rats of more dominant status; indicating that social drive is

towards the higher positions of the social structure. This being true, the level of aggression exhibited by a particular animal is an indicator of the relative level of social drive. The question remains, however, whether pharmacological manipulation of overt aggression may modify an animal's relative position within the hierarchical structure. This aspect of behavioural pharmacology has received scant attention in the literature. Indeed, in very few studies has the pharmacological manipulation of rodent rank order been attempted. Poshivalov (1979) suggested that psychotropic drugs of different classes (caffeine, diazepam, droperidol and mescaline) have different effects on the stability of dominant/subordinate relationships in mice, and that prolonged administration of drugs which suppress the level of aggression exhibited by dominant mice results in an inversion of the hierarchical structure. Thus the reduction in social drive induced as a result of decreased aggression is manifest as a reduction in rank position. Conversely, Malatynska and Kostowski (1984) demonstrated that chronic treatment of dominant rats with the antidepressants imipramine, amitriptyline, desipramine, mianserin and clomipramine per se increased the RANK SEPARATION (as determined by the respective duration of eating time) between paired rats. Similarly, chronic treatment with non-antidepressant drugs, i.e. yohimbine, amphetamine and pimozide, also increased the dominance-subordination relationship.

Previous studies have demonstrated that chronic treatment with antidepressants results in increased aggressive behaviour (see section 5.5.3), which, it is suggested, is indicative of increased social drive. The following studies were performed to examine the effect of chronic treatment with clomipramine or mianserin, at doses

which demonstrably increase aggression, on the relative rank position of a particular animal within a social group.

7.2 Methods

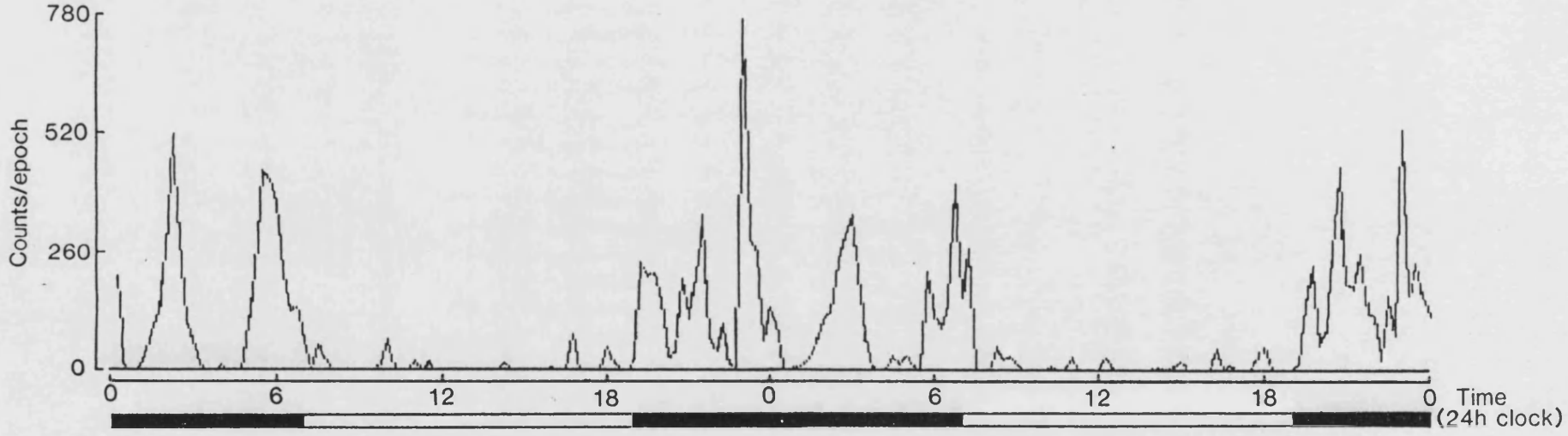
7.2.1 Experimental Design

7.2.1.1 Methodological considerations

Grant and Chance (1958) demonstrated that groups of 3 to 5 rats exhibited stable linear social hierarchies. To ease the logistics of monitoring the social behaviour of grouped animals it was decided to observe the behaviour of rats maintained in groups of 3 (i.e. triads).

In monitoring the social behaviour of grouped animals it is important that the test situation optimizes the level of social interaction to enable sufficient data to be collected to withstand critical statistical analysis. Rats are nocturnal and observations of their circadian locomotor activity throughout the light/dark phase (Fig 7.1) indicate that while such animals are quiescent during the light phase, locomotor activity occurs immediately at the onset of darkness. To enable more than one group of animals to be observed on any one day the groups of rats were housed in environmental cabinets so that the onset of the dark phase for each group could be controlled. However, each cage had to be removed from the environmental cabinet and placed in the recording cabinet to allow the recording of social behaviour on to video tape for analysis at a later date. This resulted in the subject animals exhibiting exploratory activity of the recording cabinet for at least 30 min following the change of environment. Subject animals were therefore allowed to acclimatize to the recording cabinet during the last 60

Figure 7.1 Circadian locomotor activity of grouped rats monitored continuously over 48h.
Vertical axis: locomotor activity counts per 15 min epoch for grouped rats (N=3). Horizontal axis: time in hours (24h clock). Rats were housed under a 12h/12h light-dark cycle (lights on 0700, lights off 1900). Black band indicates dark period.



Light/Dark Cycle (12:12, Lights on 0700)

min of the light phase, by which time they were invariably quiescent, prior to the room lights being turned off at the start of the dark phase. Pilot observations of triads (data not shown) indicated that during the initial 30 min of the dark phase periods of intense social interaction occurred involving all animals in the group. By 40 min, however, the social interaction was replaced by increasing periods of exploration, grooming and consummatory behaviour. Observations to assess the rank order of caged rats were therefore performed on groups of 3 rats at the onset of the dark phase for a 30 min period.

Pilot studies employed the method of Grant and Chance (1958), i.e. incidence of full submissive posture, to identify the dominant and subordinate animal in each social interaction. It was readily apparent, however, that the full submissive posture occurred only very rarely (data not shown); subordinate animals were more likely to exhibit flight-escape behaviour. The method of identifying the dominant and subordinate animals within a particular social interaction was therefore reappraised (see section 7.3).

7.2.1.2 Subjects

2 weeks after weaning groups of 3 male Wister rats were taken from stock and housed in light- and sound-attenuating environmental cabinets under a 12h:12h light-dark schedule for 2 weeks prior to each experiment (see sections 4.2 and 4.3.1), with standard laboratory chow (Labsure CRM diet) and water available ad libitum.

7.2.2 Basic Methodology

The animals, still in their home cage, were removed from the environmental cabinet 1h before the onset of the dark phase and the cage positioned inside the behavioural recording cabinet (see section 4.4.1 for description). At the start of the dark phase the room lights were turned off and the behaviour of the animals recorded under low intensity (2 lux illumination) red light on video tape, via an infrared camera positioned immediately above the recording cabinet, for the following 30 min. for analysis at a later date. Immediately following the recording period the cage was replaced in the environmental cabinet.

7.2.2.1 Preliminary observations on the stability of the hierarchical structure of rats maintained in triads

In this series of experiments the social behaviour of 3 groups of 3 rats per group was recorded from the onset of the dark phase on 3 successive days of the week (Mon-Wed) over 6 consecutive weeks. During the week prior to the experiment each group cage was placed in the recording cabinet, under those conditions described above (section 7.2.2), on 3 successive days to allow habituation to the recording apparatus. None of the animals within any group received any drug treatment.

7.2.2.2 Effect of chronic drug or drug-vehicle treatment on the rank position of sub-dominant rats

In these experiments the social behaviour of the grouped rats was monitored according the scheme indicated in Table 7.1.

Week	Experiment day (unless otherwise indicated) Mon	Tue	Wed
1 + 2	Acclimatization to recording environment		
3	-8	-7	-6
4	-1	implant pumps	D1
5	D6	D7	D8
6	D13	D14 (remove pumps)	+1
7	+6	+7	+8

Table 7.1 Experimental scheme indicating the recording sessions and operating days for the implantation or removal of mini-osmotic pumps.

-8, -7, -6, -1 : Pre-implantation recording sessions.

D1, D6, D7, D8, D13, D14 : Recording sessions following implantation.

+1, +6, +7, +8 : Recording sessions following removal of osmotic mini-pumps.

The hierarchical positions of the rats within each group were assigned according to the rank order obtained on the day immediately prior to the subcutaneous implantation of the mini-pumps, i.e. day -1, week 4. Only those animals in the subdominant position received pumps containing drug (clomipramine, 10 umol Kg^{-1} day^{-1}, or mianserin, 0.33 umol Kg^{-1} day^{-1}) or vehicle (H_2O). Rats in the dominant or subordinate rank positions were sham-operated and received a subcutaneous implant of a sterilised exhausted pump recovered from a previous control experiment where H_2O was the control vehicle used. Implantation of the pumps was carried-out according to the method described in section 4.5, following which all animals were returned to their respective environmental cabinets. Immediately after the recording session on treatment day 14 (i.e. D14, week 6) all animals were anaesthetised and the mini-pumps removed; the animals were then returned to their respective environmental cabinets.

7.3 Assessment of the hierarchical structure of rats

Chance and Silverman (1964) suggest the establishment and maintenance of rank order between caged rats is dependent on the degree of overt aggressive behaviour exhibited by each group member. Measurement of the individual elements of aggressive behaviour (as used in the social interaction studies, section 5.1.1), however, is not applicable to the assessment of rank position for each animal since the data contains no information regarding the relationship of aggressive behaviour to the overall level of dominance attained during social interaction. The level of dominance may only be determined by considering the proportion of success in terms of the total number of social interactions (Craig, 1986), and thus

definitions of dominance that stress the results of agonistic encounters are of greater heuristic value (Benton, 1982). Thus in these experiments only those social interactions which resulted in one animal being demonstrably identified as the "winner" and the other the "loser were recorded. Interactions where no discernible winner or loser could be identified were ignored. Grant and Chance (1958) used similar criteria for identifying the winner and loser of a single social interaction by noting the incidence of full submissive posture exhibited by the less dominant rat. Such postures indicate the end point of the flight-submit behavioural pathway (Fig. 7.2), but animals observed in the pilot studies of this investigation very rarely, if at all, demonstrated the full submissive posture. Rather, animals demonstrated flight-escape behaviour in response to the aggressive posturing of a cage partner and as such were deemed to be the loser of that social interaction. In some cases animals demonstrated evasive behaviour prior to any approach by the aggressor. In these instances the result of the social interaction was recorded as long as the more dominant animal could be identified.

In both preliminary and drug studies the rank position of each group member was determined as follows. The number of wins and losses for each animal was calculated. The number of wins for each animal was then expressed as a percentage of the total number of social interactions involving that animal. The percentage value indicates the success level achieved during social interaction and the higher the success level the higher the rank position of that group member. The highest value indicates the dominant animal, designated alpha; the next highest is termed the subdominant animal, designated beta;

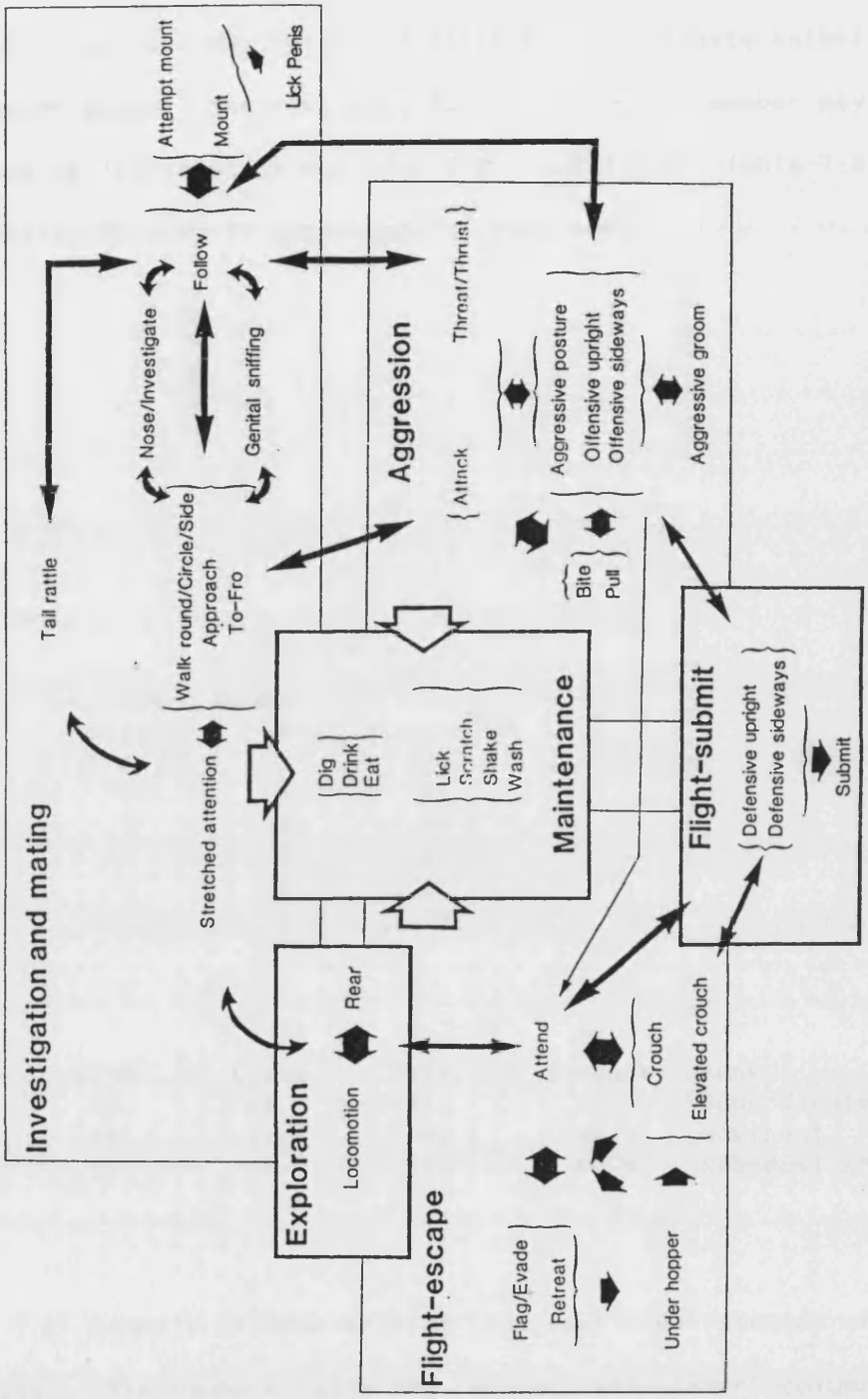


Figure 7.2 Simplified schematic representation of the pathways of social behaviour of the rat.

Individual behaviours or postures are grouped according to motivational category. Closed arrows indicate the normal progression of behaviours. Open arrows indicate displacement activity.

Adapted from Grant (1963).

and the lowest success value indicates the subordinate animal, designated gamma. The rank position of each group member may then be assigned as indicated in sections 7.3.1 and 7.3.2. Table 7.2 illustrates an example assessment of rank order.

Animal Number "Winner"	"Loser"	Score
1	2	14
2	1	31
1	3	29
3	1	37
2	3	25
3	2	19

Animal Number	Wins	Loses	Total	% Success	Rank
1	43	68	111	38.7	Subordinate
2	56	33	89	62.9	Dominant
3	56	54	110	50.9	Sub-dominant

Table 7.2 Example of data arising from rank order studies of triads. Top panel: The numbers below the "winner" and "loser" columns indicate the particular animals involved in social interaction. Bottom panel: Summary of the data to enable assignment of rank position.

7.3.1 Preliminary studies

In order to determine the stability of rank order in caged rats the rank orders for each group were assigned on the basis of the rank positions determined from the total data. Thus, at the end of the 6 week experimental period the total number of wins and losses for each animal in each group over the duration of the experiment were determined and the percentage success level calculated. Each animal within any group then held that rank regardless of the rank position indicated during any particular recording.

7.3.2 Drug studies

The hierarchical positions of the animals within each group were assigned according to the rank order obtained on day -1, week 4 (see section 7.2.2.2 and Table 7.1) and held that designation for the duration of the experiment.

7.4 Statistical analysis

7.4.1 Preliminary studies

Only three groups of animals were observed in this investigation on the hierarchical structure of triads. Since the data size is small the application of non-parametric statistical analysis is not applicable.

7.4.2 Drug studies

The non-parametric Mann-Whitney U-test was used to compare the success values for each rank position to those values obtained immediately prior to the onset of drug or drug-vehicle administration (i.e. day -1, week 4).

7.5 Results

7.5.1 Preliminary observations on the stability of the hierarchical structure of rats maintained in triads

Fig. 7.3 summarizes the mean (±sem) success values, for each recording day, of the rank positions assigned according to the hierarchical structure determined from the total data for each group. The data demonstrates that the hierarchical stucture of the 3 groups of rats maintained in triads is essentially linear in nature and stable not only from one recording day to the next but also throughout the duration of the experiment. Only during weeks 1 and 5 was the hierarchical stucture observed to deviate from the rank positions assigned from the total data. At all other recording times each animal within a particular group maintained its designated rank position. Even though only a limited number of groups were studied in this preliminary, quantitative, investigation the stability of the rank positions over time indicates that the conclusions above are broadly correct.

In the light of these observations the groups observed in the drug studies were allowed 2 weeks of acclimatization to the recording apparatus.

7.5.2 Effect of chronic drug or drug-vehicle treatment on the rank position of sub-dominant rats.

Table 7.3 and Fig. 7.4 summarize the hierarchical structure, in terms of the success values for each rank position assigned immediately prior to chronic drug or drug-vehicle treatment, for each treatment group throughout the duration of the experiment.

Figure 7.3 Stability of the hierarchical structure of grouped rats. Columns indicate the mean (and sem) success values for each rank position assigned according to the hierarchical structure determined from the total data for each group. The assigned rank positions were then used to determine the mean success level for each rank position on each recording day regardless of the actual rank position attained by an individual rat on that day.

Alpha, dominant; beta, subdominant; gamma, subordinate.

N=3 animals per group.

The behaviour of grouped animals was recorded 3 days a week (Mon, monday; Tue, tuesday; Wed, wednesday) over a 6 week period (Week 1 to Week 6).

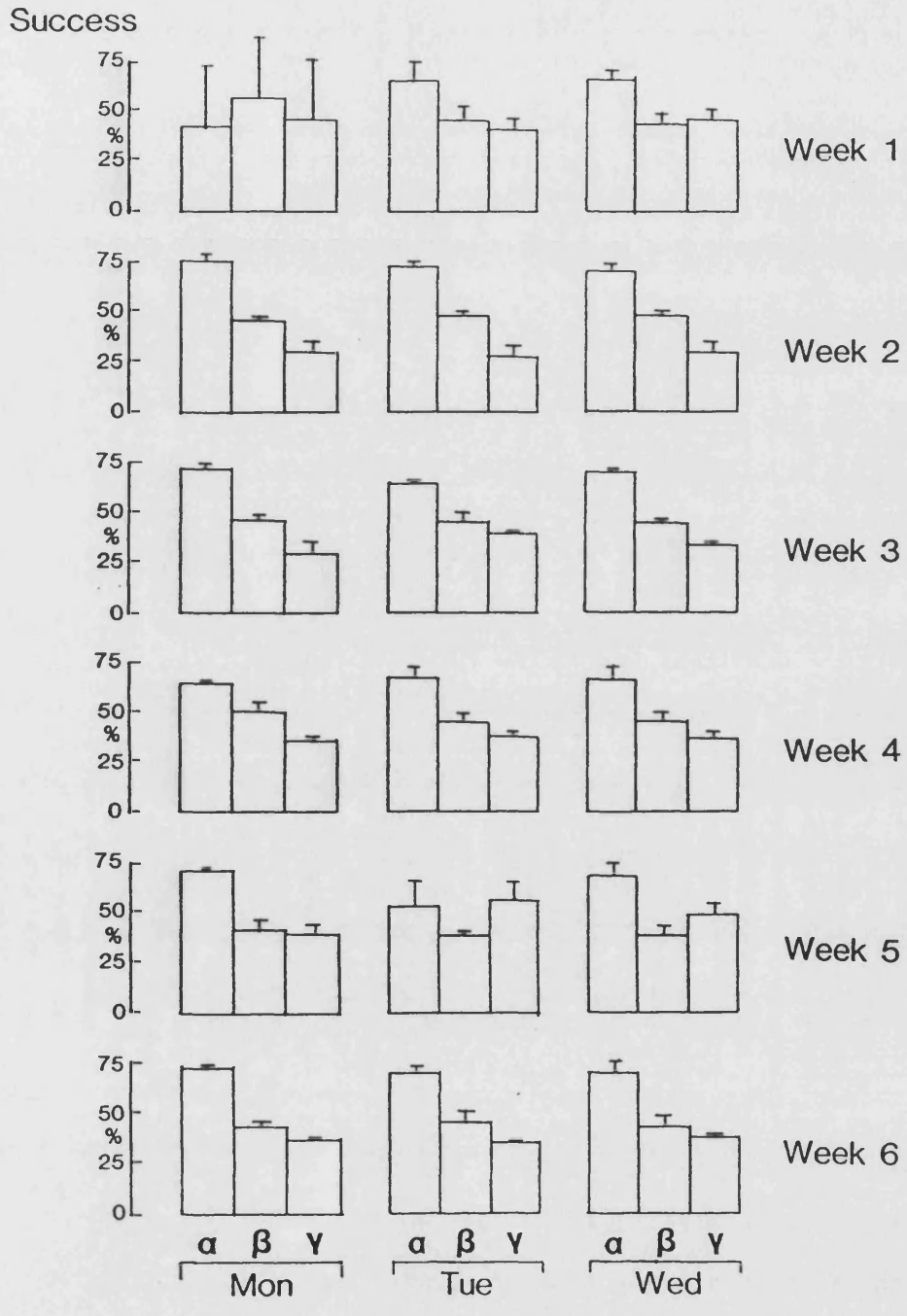

Success
75
50
%
25
0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
α β γ
Mon
α β γ
Tue
α β γ
Wed

Table 7.3 Effect of chronic clomipramine or mianserin treatment of subdominant group members on the social hierarchy of rats maintained in triads.

Hierarchical structure: Rank positions were assigned according to the success values obtained on day -1; alpha, dominant; beta, subdominant; gamma, subordinate.

Values indicate mean (± sem) success.

N=5 groups per treatment.

Treatment schedule.

-7, -6, -1 : Days pre-implantation

D1, D6, D7, D8, D13, D14 : Days of drug treatment

+1, +6, +7, +8: Days post-treatment

Subdominant rats: H_2O, 9.72 ul day^{-1} sc (N=2) and 9.68 ul day^{-1} sc (N=3); clomipramine, 10 umol Kg^{-1} day^{-1} sc; mianserin, 0.33 umol Kg^{-1} day^{-1} sc.

Dominant and subordinate rats were sham operated.

* Duration of treatment.

MWUT: a, $p<0.05$; b, $p<0.01$; compared to values on day -1.

c, $p<0.05$; d, $p<0.01$; compared to values on day +1.

		Success values (%)		
Day	Rank	H_2O	Clomipramine	Mianserin
	alpha	63.8±5.5	67.6±2.4	69.0±4.2
-7	beta	50.6±2.5	49.0±2.4	50.2±2.3
	gamma	34.4±5.9	30.4±4.2	37.0±1.5
	alpha	69.2±2.5	69.6±3.8	69.6±3.9
-6	beta	49.6±2.7	48.8±2.4	48.0±2.8
	gamma	25.4±4.4	29.8±4.7	35.2±2.4
	alpha	69.4±3.1	72.0±4.3	67.8±3.2
-1	beta	48.4±2.6	48.4±3.5	49.8±2.2
	gamma	30.8±4.6	30.8±2.7	35.0±1.4
	alpha	71.2±5.5	76.8±2.9	71.6±2.8
D1	beta *	54.4±2.8	44.0±2.2	49.0±3.2
	gamma	24.2±4.6	28.4±3.5	29.2±1.0
	alpha	68.0±3.0	56.0±1.7b	56.0±1.0b
D6	beta *	52.2±1.2	57.4±2.1a	58.2±1.4a
	gamma	33.0±2.1	36.0±3.6	33.6±2.1
	alpha	67.0±2.7	60.6±2.4a	54.4±3.2a
D7	beta *	54.6±2.2	60.4±2.6a	58.6±1.0b
	gamma	26.6±3.7	28.8±6.2	30.8±3.2
	alpha	66.0±2.7	59.2±2.0a	56.2±1.7a
D8	beta *	54.6±0.6	58.4±1.1a	56.6±1.6a
	gamma	29.4±1.4	29.0±3.1	29.6±4.8
	alpha	67.6±2.6	56.4±1.7b	55.0±2.4b
D13	beta *	54.0±1.9	61.4±4.5a	60.4±1.5b
	gamma	27.4±1.6	32.4±2.4	31.4±2.1
	alpha	66.2±2.3	56.0±0.5b	54.8±0.9a
D14	beta *	54.2±2.2	61.8±3.4a	55.6±1.4a
	gamma	31.2±1.7	31.6±3.4	33.4±2.9
	alpha	66.0±4.8	55.8±2.4b	54.0±0.7b
+1	beta	53.4±1.7	56.8±2.9a	57.0±4.0a
	gamma	27.2±4.2	35.4±3.3	27.2±4.7
	alpha	67.4±4.9	68.0±2.4d	61.0±1.1c
+6	beta	55.0±2.2	49.6±2.5c	48.8±3.0c
	gamma	29.2±3.5	24.6±7.5	25.8±5.2
	alpha	69.6±3.2	68.8±2.8d	63.3±2.5d
+7	beta	53.0±2.4	44.4±3.0d	50.0±1.4c
	gamma	30.0±1.7	33.4±3.2	33.5±1.8
	alpha	68.0±2.9	67.2±3.5d	62.0±2.0d
+8	beta	53.0±2.1	45.4±2.1d	47.8±1.6c
	gamma	29.8±2.6	31.8±4.9	30.0±3.2

Figure 7.4 Effect of chronic treatment with clomipramine or mianserin of subdominant group members on the hierarchical structure of rats maintained in triads.

Hierarchical structure: Rank positions were assigned according to the success values obtained on the day immediately preceding implantation of the mini-pumps (i.e. day -1, week 4; see Table 7.1).

Alpha, dominant; beta, subdominant; gamma, subordinate.

Columns indicate mean (± sem) success.

N=5 groups per treatment.

For treatment schedule see Table 7.1 and legend to Table 7.3.

Pre 7, pre-treatment day 7 (i.e. -7, week 3).

Pre 1, pre-treatment day 1 (i.e. -1, week 4).

Drug 1, drug treatment day 1 (i.e. D1, week 4).

Drug 7, drug treatment day 7 (i.e. D7, week 5).

Drug 14, drug treatment day 14 (i.e. D14, week 6).

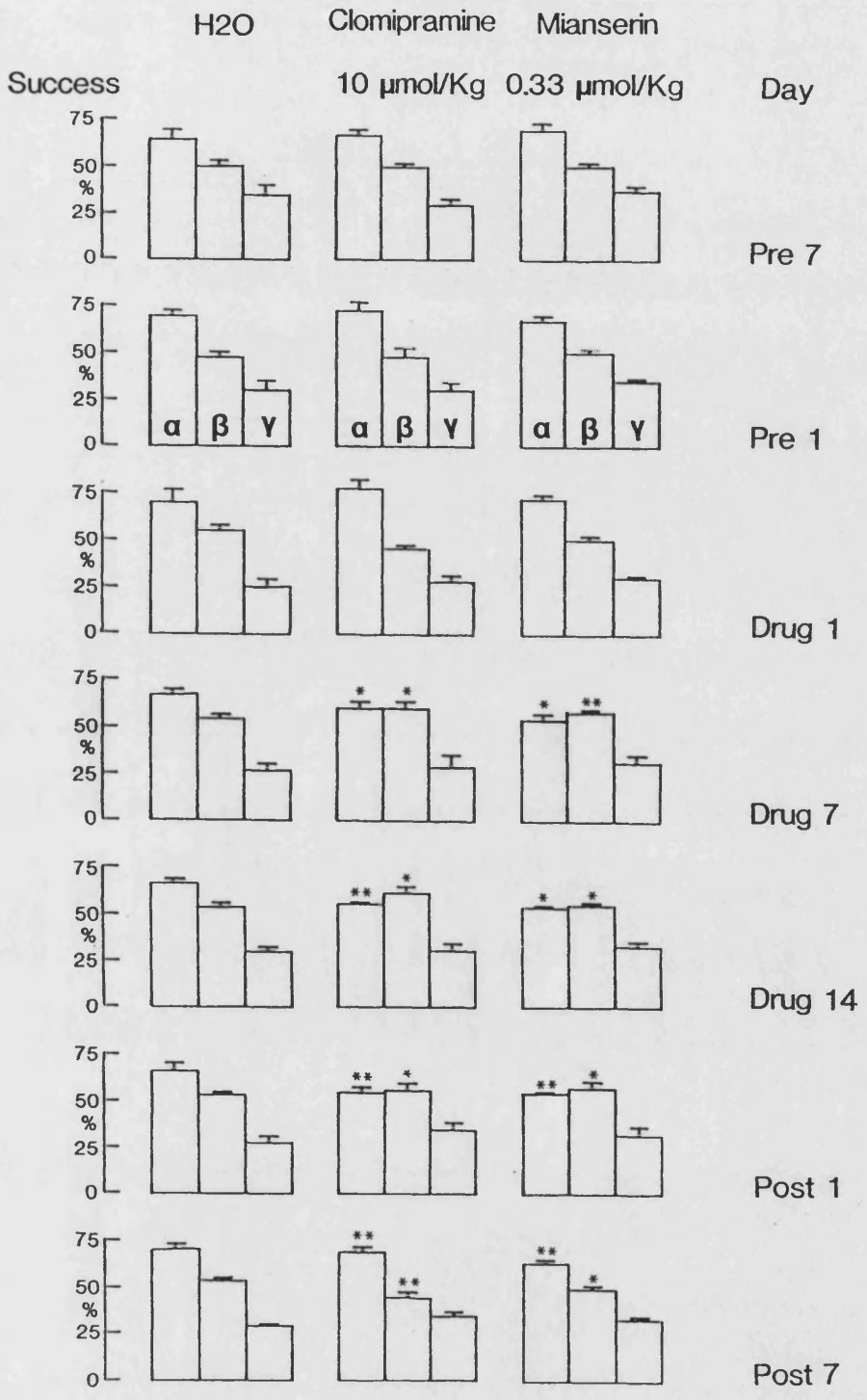
Post 1, post-treatment day 1 (i.e. +1, week 6).

Post 7, post-treatment day 7 (i.e. +7, week 7).

Dominant and subordinate rats were sham operated.

MWUT: Drug 7, Drug 14 and Post 1; * $p<0.05$, ** $p<0.01$; compared to values on day Pre 1.

Post 7; * $p<0.05$, ** $p<0.01$; compared to values on day Post 1.


H2O
Clomipramine
Mianserin
Success
10 µmol/Kg
0.33 µmol/Kg
Day
75
50
%
25
0
α
β
γ
Pre 7
Pre 1
Drug 1
Drug 7
Drug 14
Post 1
Post 7

In those groups where the subdominant animals were treated chronically with water no change in the social hierarchy of the triads was observed throughout the duration of the experiment. Likewise, the social hierarchy of those groups where the subdominant animals received chronic clomipramine or mianserin treatment demonstrated little disruption in the hierarchical structure at day 1 of drug treatment. By day 6, however, subdominant rats treated chronically with either clomipramine or mianserin exhibited significant increases in their success levels, from 48.4±3.5% and 49.8±2.2% at day -1 to 57.4±2.1% and 58.2±1.4% respectively ($p<0.05$ in both cases), concomitant with significant decreases in the success level of the respective Sham-operated dominant rats, from 72.0±4.3% and 67.8±3.2% at day -1 to 56.0±1.7% and 56.0±1.0% respectively ($p<0.01$ in both cases). The observed modification of the social hierarchy was maintained throughout the period of drug treatment and was still present at day 1 following the cessation of drug administration. Little or no change was observed in the success level of Sham-operated subordinate animals where the subdominant group member received chronic clomipramine or mianserin treatment. By day 6 following drug treatment the hierarchical structure for each group had returned to that determined prior to the onset of drug administration.

7.6 Discussion

Housing rodent colonies in closed communities (where the group members are in continuous social intercourse) allows a hierarchical structure to develop between the members of the group. In order to determine the rank positions of each group member the social behaviours pertinent to the maintenance of the hierarchy must be observed and recorded. As with the majority of ethological experiments, the major problem is to ensure that the animals will perform at the time required by the observer. In these studies the social behaviour of triads of rats were observed during the initial 30 min of the dark phase of the light-dark cycle since during this time intense social interaction occurs involving all animals within the group. Such behaviour was observed to occur ritualistically at the onset of every dark phase and performs the function of maintaining the social structure of the group.

Preliminary observations of the social structure of rats grouped in triads indicated that the nature of the hierarchical structure is both linear and stable over the six week observation period. Such observations are in accord with those of Grant and Chance (1958). The magnitude of the data arising from these studies and the stability of the success values obtained for each rank position indicate that the hierarchical structure of rats may well provide a suitable robust animal model in which to study the effects of psychotropic drugs on social structure.

Chance and Silverman (1964) suggest that the level of aggression exhibited by individual animals is an integral component in the development of hierarchical structure between male rats. In

addition, Poshivalov (1979) demonstrated that reducing the level of overt aggressive behaviour in mice by chronic treatment with psychotropic drugs results in the disruption of the hierarchical structure. These observations indicate that overt aggressive behaviour exhibited by group members plays an important role in the manifestation of social drive. Furthermore, chronic antidepressant treatment has previously been shown to increase the dominance-subordination relationship between paired rats (Malatynska and Kostowski, 1984). With the exception of the two studies cited above this area of ethology has received scant attention by behavioural pharmacology; for example, there is no published data on the effect of increased aggressive behaviour exhibited by a non-dominant group member on the hierarchical structure of that group.

Earlier studies in this investigation (see sections 5.5.2 and 5.5.3) demonstrated that chronic, but not acute, treatment with examples of antidepressants, but not the antipsychotic haloperidol nor the anxiolytic diazepam, increased aggressive behaviour exhibited by resident rats during social interaction with unknown intruder conspecifics. Since similar chronic treatment regimes had no effect on the level of exploratory locomotor activity exhibited by rats (see section 6.4.2) it was suggested that the increased level of aggressive behaviour was indicative of increased social drive rather than increased basal activity. In these studies chronic treatment of subdominant rats with clomipramine or mianserin, at doses known to increase aggressive behaviour, increased the relative rank position of the treated animals at the expense of the level of dominance enjoyed by the dominant group member. Inversion of the relative rank

positions for the dominant and drug-treated subdominant did not occur, however, rather both animals exhibited parity of rank position. The rank position of subordinate group members was not affected by drug treatment of the subdominant rats. By comparison, the hierarchical structure of those groups of rats where the subdominant member received chronic treatment with water (drug-vehicle) remained constant throughout the duration of the experiment. The modification of the social structure induced by clomipramine or mianserin was dependent on chronic treatment since no effect was observed on day 1 of treatment and was still present on day 1 following the cessation of drug treatment. However, the pre-treatment hierarchical structure was recovered by day 6 post-dose. The time-course of the clomipramine- or mianserin-induced modification of social hierarchy closely correlates to that observed in the social interaction studies. The effects of chronic clomipramine or mianserin treatment on the rodent social hierarchy therefore appear related to their ability to induce overt aggressive behaviour.

The results of this study support the suggestion of Grant and Chance (1958) that animals in lower rank positions are attracted towards the higher ranks of the social structure; thus the direction of social drive is towards the more dominant ranks. In addition, the level of aggressive behaviour is an important component in determining the relative rank position of individual animals (as suggested by Chance and Silverman, 1964).

In conclusion, therefore, the results of this investigation support the suggestion that the hierarchical structure of rodents provides a

suitable animal model by which the effects of psychotropic drugs on social drive may be examined. In addition, the data indicate that chronic treatment with the antidepressants clomipramine and mianserin increases social drive, which, in rats at least, is manifest as increased aggressive behaviour. In the social interaction studies increased aggression was also observed following chronic treatment with the antidepressants fluoxetine, iprindole and phenelzine. In the light of these studies such drug-induced change in rodent behaviour may also be indicative of increased social drive.

The relationship of these results to the general effects of acute and chronic treatment with antidepressants on rodent social behaviour and their implications with respect to published investigations by other workers are discussed in detail in chapter 8.

CHAPTER 8 DISCUSSION

CHAPTER 8 DISCUSSION

Before attempting to evaluate the relevance of the effects of acute and chronic antidepressant treatment on the social behaviour and exploratory locomotor activity of rats, in the broad context of the current theories on the clinical mode of action of antidepressants and the aetiology of depression, it may be useful to summarise the aims and major conclusions of these studies.

The investigations described in chapters 5, 6 and 7 follow a logical progression of thought.

The aim of the social interaction studies, described in chapter 5, was to compare the acute and chronic effects of psychotropic agents (principally examples of antidepressants) on the endogenous patterns of social behaviour of rats.

The results indicated that acute and chronic antidepressant treatment have diametrically different effects on endogenous patterns of social behaviour in rats.

Acute treatment with the antidepressants clomipramine, fluoxetine, iprindole, mianserin or phenelzine decreased aggressive behaviour exhibited by resident rats but concomitantly increased flight behaviour at doses which had little or no effect on the total number of behavioural elements observed during social interaction with an unknown conspecific. The antipsychotic haloperidol or the anxiolytic diazepam also reduced aggressive behaviour but only at doses which concomitantly decreased the total number of behavioural elements observed. Acute treatment with haloperidol also concomitantly

reduced flight-submit behaviour while diazepam concomitantly reduced environmental exploratory behaviour.

The broad effects on social behaviour observed following acute treatment with haloperidol or diazepam suggested that such effects may be due to the onset of overt sedation. The data therefore implied that the selective effects on aggressive behaviour observed following acute antidepressant treatment may possibly be indicative of a specific effect on rodent social behaviour (i.e. reduced social drive). At this stage of the investigation this suggestion was somewhat tentative for the following reason: the aggressive elements of rodent social behaviour occur at the later stages of the behavioural pathways (Grant, 1963) and thus aggressive postures may simply be more sensitive to the sedative effects of psychotropic compounds. Such data per se may not therefore suggest that acute treatment with antidepressants has a specific effect on rodent social behaviour. Exploratory locomotor activity of rodents is known to be sensitive to the sedative effects of psychotropic agents (Brown et al., 1985) and this animal model was employed to assess the sedative potential of these compounds.

Chronic treatment with the antidepressants, but not haloperidol or diazepam, at minimal effective doses on social behaviour when given acutely, increased the overt aggressive behaviour exhibited by resident rats without consistently affecting the total number of behavioural elements observed during social interaction. It was suggested that this change in rodent social behaviour may be indicative of increased social drive. This explanation was further examined by observing the effect of clomipramine or mianserin,

administered chronically to subdominant rats, on the general hierarchical structure of grouped rats. An alternative explanation, however, was that such drug treatment simply increased the basal level of activity of the treated rats. This possibility was addressed by monitoring the exploratory locomotor activity rats following chronic treatment with the antidepressants or haloperidol.

The aim of the exploratory locomotor activity studies, described in chapter 6, was to assess the sedative potential of the antidepressants and haloperidol and diazepam following acute treatment. In addition, the ability of chronic antidepressant or haloperidol treatment to modify the basal level of exploratory locomotion of rats was examined.

Acute treatment with haloperidol and diazepam, but not the antidepressants, reduced exploratory locomotor activity at doses previously demonstrated to reduce both aggressive behaviour and the total number of behavioural elements exhibited by resident rats during social interaction. Since the doses of haloperidol and diazepam used in these studies were demonstrably sedative, it was concluded that the effect of these two compounds on the social behaviour of resident rats was due to the onset of overt sedation. Both haloperidol and diazepam exhibited equi-potent effects in their ability to reduce exploratory locomotor activity and the total number of behaviours observed during social interaction. Thus measurement of the total number of behaviours provides identical information regarding the sedative potential of compounds as the measurement of exploratory locomotion. The social interaction test therefore provides an inbuilt control for the incidence of overt sedation.

The reduction in aggressive behaviour observed following acute treatment with the antidepressants therefore appears to be a specific effect on rodent social behaviour.

Chronic treatment with either the antidepressants or haloperidol had no effect on the basal level of exploratory locomotor activity of rats. The increase in aggressive behaviour exhibited by resident rats during chronic antidepressant treatment therefore was not related to an increase in basal activity but, possibly, indicative of increased social drive. Whether such an effect could be attributed to increased social drive was examined in a more relevant animal model by studying the effect chronic treatment of subdominant rats with clomipramine or mianserin on the hierarchical structure of grouped rats.

The investigation involving assessment of the hierarchical structure of rats was designed under the premise that the rank position of an individual group member was indicative of that animals' level of social drive. If the increase in overt aggressive behaviour exhibited by resident rats during chronic antidepressant treatment in the social interaction test was a manifestation of increased social drive then similar drug treatment should increase the relative rank position of an individual animal within a social group. The primary aim of this study, described in chapter 7, was therefore to examine the effect of chronic antidepressant treatment on a particular group members rank position.

Chronic treatment with clomipramine or mianserin increased the apparent success level of subdominant rats during social interaction

with the conspecific group members at the expense of the success level exhibited by dominant rats. The subdominant animals receiving chronic drug treatment did not become the most dominant group member, rather such animals achieved parity of rank position with the formally dominant members. This study indicates therefore that the increased aggressive behaviour induced by chronic antidepressant treatment is indicative of increased social drive.

In conclusion, the diametrically different effects of acute and chronic antidepressant treatment on the endogenous patterns of social behaviour of resident rats are indicative of decreased and increased social drive respectively, which, in this species at least, is manifest by the appropriate changes in overt aggressive behaviour.

Moyer (1968) described 7 different forms of aggressive behaviour dependent on the current environmental and social situation (see also Eichelman, 1978).

1 Predatory; aggressive behaviour is evoked by the presence of a natural object of prey, e.g. frog-attack and possibly muricide (mouse-killing) and filicide (pup-killing) by adult rats (Baenninger, 1978; Blanchard and Blanchard, 1977).
2 Intermale; aggressive behaviour directed at a male conspecific to which the attacker has not become habituated, e.g. between male conspecifics in the social interaction test and possibly muricide in rats. Also termed conspecific attack (Blanchard and Blanchard, 1977).
3 Fear-induced; usually preceded by attempts to escape and is dependent on the degree of confinement of the defensive animal

(also described as defensive aggressive), e.g. aggression exhibited by rats during non-evasive footshock and aggressive behaviour exhibited by intruder rats following investigation by resident conspecifics during social interaction.

4 Irritable; the stimulus for this type of aggressive behaviour is the presence of any attackable object or organism. Muricide, filicide and shock-induced aggression in rats may be included in this category.

5 Territorial; occurs in an area in which the animal has established itself. The territorial stimulus is essential to this aggressive behaviour and its intensity decreases as the attacking subject gets further away from its own territory or becomes habituated to the intruder. For example, isolation-induced aggression exhibited by resident rats in the social interaction test.

6 Maternal; usually exhibited by females in defence of the young.

7 Instrumental; demonstrated by an increase in the tendency to engage in destructive behaviour when that behaviour has been reinforced in the past, e.g. shock-induced aggression in rats.

Aggressive behaviour may be categorised more simply into Affective and Predatory aggression depending on the incidence of accompanying marked sympathetic arousal (Eichelman, 1978; and references cited therein).

The aggressive behaviour exhibited by resident rats following short-term isolation and directed at the conspecific intruder in the social interaction test is a composite of inter-male and territorial aggressive behaviour (since social interaction occurs between male conspecifics and in the resident animals' home cage). Such

aggressive behaviour has been termed affective aggression (Moyer, 1968; Eichelman, 1978) since it is associated with pronounced sympathetic arousal. Moyer's classification of aggressive behaviours are not therefore mutually exclusive. While aggression may not be a unitary concept (Moyer, 1968; Bernstein and Moyer, 1970) the possibility exists that the different forms of aggressive behaviour differ only in their intensity and are dependent on the experimental situation (see Rodgers and Brown, 1976), implying that the underlying neuronal substrates mediating aggressive behaviour are common to each or at least inter-related.

The neuronal substrates controlling aggressive behaviour in the cat have been well reviewed by Clemente and Chase (1973). While the phylogenetic differences between the cat and rat are readily accepted both animals exhibit analogous behaviours related to aggression (both offensive and defensive) and flight in addition to homologous brain centres and neuronal circuits. It would not be unreasonable to assume some degree of similarity between the central systems controlling aggression in the cat to those systems in other vertebrates including the rat and man.

Electrolytic lesions of the amygdala reduce both shock-induced aggression (Anand et al, 1977; Eichelman, 1971; Vochteloo and Koolhaas, 1987; where fighting, exhibited as stereotyped boxing behaviour, occurs between paired rats on electrical stimulation of the cage floor), and the level of social interaction and dominance in rats (Bunnell, 1966). In studies where more specific electrolytic lesions of the amygdaloid complex were produced Zagrodzka and Fonberg (1978) demonstrated that electrolytic lesions

of the ventromedial, but not dorsolateral, amygdala abolished predatory behaviour in cats. In addition, an extensive study (Miczek et al., 1974) demonstrated that electrolytic lesions of the periamygdaloid cortex, cortical amygdaloid nucleus or bed nucleus of the stria terminalis (the dorsal amygdaloid efferent pathway), but not lesions of the lateral or central amygdaloid nuclei, reduced or eliminated attacks or signs of dominance in fights induced by social isolation and food competition in rats, but had little or no effect on shock-induced aggression or muricide. Conversely, only lesions of the lateral or central nuclei reduced shock-induced aggression. These observations are in contrast to those of Horovitz et al. (1966), who had previously shown that centromedial lesions of the amygdala blocked muricide behaviour in rats, but in agreement with the experiments of Bermond (1982), where lesions of the stria terminalis and its bed nucleus resulted in reduced conspecific aggression. The findings of Miczek et al. (1974) suggest that decreased conspecific aggression may be ascribed to cortical nucleus damage, however their results may also reflect attenuated flight behaviour since Kemble et al. (1984) demonstrated that lesions in the medial area of the amygdala which spared the medial nucleus resulted in reduced flight behaviour of wild rats, while those lesions which included damage to the medial nucleus reduced defensive behaviour. Taken together these results indicate that the amygdala is intimately involved in the mediation of aggressive, flight and defence systems and that within the amygdala there is a high level of differential organisation of these behaviours.

Specific areas of the hypothalamus are also involved in the modulation of aggressive behaviour. Electrolytic lesions of the

lateral hypothalamus reduces fighting in rats (Anand et al., 1977), while electrical stimulation induces predatory attack in cats (Hutchinson and Renfrew, 1966; Proshansky and Bandler, 1975; Shaikh et al., 1984) and intraspecific aggression in rats (Mos et al., 1982), and stimulation of the ventrolateral hypothalamus elicits both muricide and filicide in rats (Woodworth, 1971). Proshansky and Bandler (1975) also demonstrated that electrical stimulation of the midbrain tegmental area mimicked, but unilateral electrolytic lesions of the mid-brain attack sites abolished, predatory attack resulting from hypothalamic stimulation. These results indicate a hypothalamic-midbrain interaction via the medial-forebrain bundle. In addition, Shaikh et al. (1985) showed that the predatory behaviour of cats induced by lateral hypothalamic stimulation may be suppressed by stimulation of the medial aspect of the tegmentum but facilitated by stimulation of the lateral tegmentum. Lesions of the ventromedial hypothalamus have been shown to increase shock-induced aggression in rats (Eichelman, 1971), which is supported by the observations of Olivier (1977) who demonstrated that rats with anterior ventromedial hypothalamic lesions exhibited increased defensive aggressive behaviours, similar to those behaviours seen in the shock-induced aggression test, while rats with posterior ventromedial hypothalamic lesions exhibited increased offensive intermale aggression. These results indicate that the anterior and posterior ventromedial hypothalamic areas exert inhibitory control over defensive and offensive aggressive behaviours respectively. Conversely, electrical stimulation of the ventromedial hypothalamus of cats has been shown to induce affective defensive aggressive behaviour in cats (Pott et al., 1987; Shaikh et al., 1985). The work of Pott et al. (1987) suggests that

behaviour induced by stimulation of the ventromedial hypothalamus may be modulated by the central gray such that the dorsal aspect suppresses affective defence while the ventral region facilitates such apparently aggressive behaviour.

Electrolytic lesions of the septum and hippocampus have been demonstrated to increase shock-induced fighting in rats (Eichelman, 1971). The increase in shock-induced aggression following septal lesions is supported by the experiments of Anand et al. (1977).

Other areas within the CNS are also involved in maintaining the integrity of the aforementioned structures pertinent to the modulation of aggressive behaviour. The main and accessory olfactory bulbs send ipsilateral projections to the cortical amygdaloid region (Macrides and Davis, 1983). Surgical olfactory bulbectomy has been demonstrated to result in degenerating fibres in the anterior hippocampus, the corticomedial nuclei of the amygdala, the bed nucleus of the stria terminalis and the pre-optic area of the hypothalamus associated with a series of behavioural changes characterised by hyperactivity and hyper-reactivity (Cairncross et al., 1979). Behaviourally, olfactory bulbectomy of rats has been shown to induce muricide (Malick, 1976).

The amygdala, hypothalamus and septum also receive projections from the dorsal and medial raphe (Steinbusch and Nieuwenhuys, 1983). Electolytic lesions of both the dorsal and median raphe induce muricide in rats (Grant et al., 1973) concomitant with reduced 5-HT function in the forebrain. The dorsal and median raphe exercise synergistic inhibitory control over muricide behaviour since if only

one of the nuclei is lesioned then muricide does not occur; it is only when both nuclei are lesioned that muricide is induced (Vergnes, 1978).

In summary, the central structures controlling aggressive behaviour appear to be functionally and anatomically related. The focal structure in aggressive behavioural patterns appears to be the hypothalamus since its destruction disrupts both spontaneous and induced aggression. Different agonistic behavioural patterns may be elicited by electrical stimulation of specific areas of the hypothalamus. Thus attack behaviour may be induced by stimulation of the lateral and dorsomedial areas; defensive behaviour by stimulation of the ventromedial area of the anterior, middle and posterior nuclei; and flight behaviour by stimulation near or within the dorsomedial nucleus (Clemente and Chase, 1973). Lesion studies indicate that these hypothalamic nuclei are interconnected such that destruction of one of these areas results in the summated expression of behaviours resulting from the remaining areas. The amygdala modulates the activity of the ventromedial neurons of the hypothalamus via the inhibitory dorsal amygdaloid efferent pathway (stria terminalis), originating from cell bodies in the corticomedial nuclei of the amygdala, and the ventral amygdalofugal pathway, originating from cell bodies of the basolateral area of the amygdala (Shiosaka et al., 1983), which is initially excitatory but then inhibitory on neurons of the hypothalamus (Clemente and Chase, 1973). The septum inhibits general agonistic patterns by suppressing aggressive behaviour of both amygdaloid and hypothalamic origin. The mid-brain then relays the information from the hypothalamic/ amygdaloid/septal axis, presumably to the brain stem and common

spinal paths for the visceral and somatic motor expression of behaviour.

5-HT, NA and DA (among other neurotransmitters) are known transmitters within the amygdala, hypothalamus and septum.

The 5-HT innervation of the amygdala arises from cell bodies located principally in the dorsal raphe nucleus while the noradrenergic and dopaminergic terminals probably originate from the locus coeruleus (LC) and ventral tegmental area (VTA) respectively. The hypothalamus receives serotonergic innervation from the median and dorsal raphe, noradrenergic input from the lateral tegmental cell bodies and dopaminergic innervation from the VTA. Lastly, the septum receives substantial noradrenergic input from both the lateral tegmental and dorsal medullary systems, and serotonergic input from the mesencephalic raphe nuclei via the ventral ascending (mesolimbic) pathway which enters the medial forebrain bundle in the lateral hypothalamus (Clemente and Chase, 1973; Cooper et al., 1978; Lindvall and Bjorklund, 1983; Shiosaka et al., 1983; Silverman and Pickard, 1983; Steinbusch and Nieuwenhuys, 1983).

The involvement of 5-HT, NA and, possibly, DA in the mediation of aggressive behaviour in rodents has also been suggested by both pharmacological and biochemical studies.

Para-chlorophenylalanine (pCPA) reduces 5-HT levels in the CNS by irreversible inactivation of tryptophan hydroxylase (Jequier et al., 1967), the rate limiting enzymic step of 5-HT biosynthesis. Administration of pCPA to rats induces predatory aggression directed

at mice (Kreiskott and Hoffman, 1975) and frogs (McLain et al., 1974) which may be so intense as to result in muricide (Berzsenyi et al., 1983; Gibbons et al., 1978a; McLain et al., 1974; Miczek et al., 1975; Paxinos et al., 1977; Rolinski, 1975b) and filicide (Copenhaver et al., 1978; Miczek et al., 1975). Muricide may also be induced following treatment with fenfluramine (Gibbons et al., 1978b) which is neurotoxic to cells of the raphe nuclei and reduces brain 5-HT (Harvey et al., 1977). Conspecific aggressive behaviour of male resident rats is also increased following pCPA administration (Rolinski, 1975b). In a study by Vergnes et al. (1986) pCPA was demonstrated to have different effects on offensive and defensive aggression. Thus resident rats treated with pCPA exhibited increased aggressive behaviour directed at a conspecific intruder but had no significant effect on the level of aggression exhibited by the intruder animals. In addition, pCPA has been shown to have either little or no effect on shock-induced aggression (which is indicative of defensive aggressive behaviour) in rats (Conner et al., 1970; McLain et al., 1974) or to increase such aggressive behaviour (Sheard et al., 1977). Furthermore, Sheard et al. (1977) also demonstrated that low doses of LSD given peripherally, which were previously demonstrated to inhibit the firing rate of raphe neurons and decrease the release of 5-HT, enhanced shock-induced aggression. Experiments examining the effect of serotonergic agents on shock-induced aggression should be treated with care since 5-HT plays an important role in the mediation of pain sensitivity. Rodgers (1977) demonstrated that direct application of 5-HT to the corticomedial amygdala decreased, while the 5-HT_2 antagonist methysergide increased, shock-induced aggression. 5-HT and methysergide were also shown to increase and decrease respectively

pain thresholds in the "flinch-jump" test. The serotonergic compounds therefore indirectly affected shock-induced aggressive behaviour by alterations in the animals sensitivity to the electroshock. Administration of pCPA has also been demonstrated to increase aggression in wild mice (Matte and Tornow, 1978) and potentiate the affective aggression exhibited by cats induced by electrical stimulation of the ventral-medial hypothalamus (Katz and Thomas, 1976). In these latter experiments the induction of offensive, but not defensive, aggressive behaviour appears to be associated with a marked reduction (by at least 80%) in the level of 5-HT in the CNS. If 5-HT is intimately involved in the modulation of aggressive behaviour then pharmacological manipulation with agents which specifically restore or increase central 5-HT activity would be expected to reverse the effects of pCPA-treatment on aggressive behaviour.

Acute treatment with 5-HTP, the immediate precursor of 5-HT, reduces pCPA-induced muricide (Gibbons et al., 1978a; Kulkarni et al., 1973; Miczek et al., 1975; Paxinos et al., 1977) and filicide (Copenhaver et al., 1978). Muricide may also be reduced by L-tryptophan, the dietary precursor of 5-HTP, and quipazine (Gibbons et al., 1978b). Quipazine is an antagonist at 5-HT terminal autoreceptors (5-HT_{1B} binding site; Engel et al., 1986) stimulation of which is thought to mediate 5-HT's inhibitory effect on its own release (Conn and Sanders-Bush, 1987). Blockade of these receptors by quipazine presumably leads to increased 5-HT release via inhibition of the negative feedback system. Furthermore, blockade of the 5-HT re-uptake system into presynaptic terminals of rat brain by fluoxetine, which results in increased synaptic concentration of

5-HT, also reduces muricidal behaviour (Berzsenyi et al., 1983; Gibbons et al., 1978b). These results suggest an inhibitory effect of 5-HT over predatory and affective aggression (i.e. offensive behaviour) but little or no effect over defensive behaviour.

Intraventricular or intracerebral administration of 6-OHDA produces an extensive depletion of brain noradrenaline and dopamine and a degeneration of central catecholamine-containing neurons (Cooper et al., 1978). Rats treated intraventricularly with 6-OHDA exhibit increased shock-induced aggressive behaviour (Sorenson and Gordon, 1975) suggesting that NA and/or DA may be involved in the expression of defensive aggressive behaviour. Conversely, McLain et al. (1974) showed that treatment of rats with alpha-methyl-para-tyrosine (aMpT), which reduces the brain concentration of both NA and DA by inhibiting tyrosine hydroxylase (the rate limiting step in the biosynthesis of the catecholamines), had little or no effect on shock-induced aggression but effectively increased the frequency of muricide. These conflicting results on shock-induced aggression may simply reflect the time-course and treatment regimes of both experiments. Sorenson and Gordon (1975) allowed a two week period to elapse before shock-induced fighting was assessed during which the chemical sympathectomy (destruction of the NA and DA nerve terminals) would allow denervative supersensitivity to develop, while McLain et al. (1974) administered aMpT over a three day period to obtain maximal depletion of central catecholamine stores. In another series of experiments, Anand et al. (1977) demonstrated that 3h pre-treatment with reserpine, at which time the degranulation induced by reserpine would cause the release of both NA and DA, reduced shock-induced fighting while acute treatment with the dopamine agonist apomorphine,

the catecholamine precursor L-DOPA and amantidine (which increases the synthesis and release of DA) all increased fighting behaviour. Conversely, acute treatment with imipramine (which blocks NA re-uptake) reduced shock-induced fighting. In accordance with these observations, Geyer and Segal (1974) observed that intraventricularly administered NA decreased, while DA increased, shock-induced fighting in rats. These results indicate a facilitatory effect of DA, but an inhibitory effect of NA, in shock-induced fighting behaviour in rats. DA has also been implicated, albeit indirectly, in the facilitation of intermale aggressive behaviour in rats. Baggio and Ferrari (1983) demonstrated that intraperitoneal administration of lisuride (a dopamine agonist) induced fighting behaviour which was blocked by pre-treatment with haloperidol (DA antagonist) but not methysergide ($5\text{-}HT_2$ antagonist).

The overall impression of recent investigations into the neurotransmitters controlling aggressive behaviour indicates that 5-HT and NA exert an inhibitory effect over aggression while DA exerts a facilitation of aggressive behaviour. However it should be remembered that other neurotransmitters, e.g. acetylcholine, GABA and numerous peptides, may also be involved in the modulation of aggressive behaviour (see Eichelman, 1979; Eichelman and Thoa, 1973; Poshivalov, 1982; Romaniuk and Golebiewski, 1977).

A reduction in central serotonergic and/or noradrenergic activity or an increase in dopaminergic activity thus invariably increases aggressive behaviour in rodents. Long term isolation of mice or rats results in marked changes in rodent behaviour (Valzelli, 1978; Spevak et al., 1973). In mice isolated for 4 weeks the behavioural

change is characterised by increased aggressive behaviour (DaVanzo et al., 1966) which appears to be associated with reduced 5-HT and NA turnover and increased DA turnover (Valzelli, 1978) even though little or no change was observed in the brain levels of these monoamines (DaVanzo et al., 1966; Valzelli, 1974). Isolation of rats from 2-3 weeks of age results in modified sexual and social behaviour by day 104-129 (Spevak et al., 1973) and by 10-12 months of isolation the complex behavioural syndrome is characterised not by increased aggressive behaviour but, in part, by elevated locomotor activity (Garzon et al., 1979). In a study by Valzelli (1974) only 40% of adult male wistar rats showed muricide behaviour after 6 weeks of isolation, while other animals from this strain exhibited either "friendly" or "indifferent" behavioural patterns towards intruder mice. Sprague-Dawley or Buffalo strains of rat showed no aggressive (i.e muricide) behaviour. This study also demonstrated that, as with mice, isolation did not modify the levels of brain amines of muricidal rats, however, 5-HT turnover was reduced but NA turnover was increased while DA turnover was unchanged (Valzelli, 1974).

The biochemical changes induced by long term isolation which results in increased aggressive behaviour thus support the argument that aggressive behaviour may be associated with reduced central serotonergic and possibly noradrenergic activity or increased dopaminergic activity.

The current series of experiments have demonstrated different effects of acute and chronic treatment with examples of antidepressants on rodent social behaviour. Thus acute treatment reduced affective aggressive behaviour at doses which were not inherently sedative,

while, conversely, chronic treatment increased aggressive behaviour. These antidepressant-induced effects on endogenous patterns of rodent social behaviour are indicative of decreased and increased social drive respectively. Likewise, it has previously been reported that acute treatment with examples of antidepressants and, in addition, amphetamine-like compounds and antihistaminics selectively reduced muricide behaviour in rats at doses which were not associated with overt sedation (Horovitz et al., 1966). Similarly Delini-Stula and Vassout (1979) demonstrated that non-sedative doses of antidepressants reduced both muricide behaviour in rats and long-term (4-8 weeks) isolation-induced aggression in mice but had little or no effect on shock-induced aggression in rats. In the same study examples of neuroleptics and anxiolytics (diazepam and oxazepam) only reduced aggressive behaviour (muricide and either isolation-induced fighting in mice or shock-induced aggression in rats respectively) at doses which were inherently sedative. Such results compare favourably with the results obtained in this study not only for the acute effects of antidepressant treatment but also for the effects of haloperidol and diazepam on rodent social behaviour. Acute treatment with antidepressants, stimulants, anticholinergics and antihistaminics have also been demonstrated to reduce muricide behaviour exhibited by both spontaneous killer rats and olfactory bulb lesion-induced killer rats (Malick, 1976), while acute treatment with fluoxetine demonstrably reduced pCPA-induced muricide behaviour in rats (Berzsenyi et al., 1983; Gibbons et al., 1978b). In contrast to Delini-Stula and Vassout (1979), Crowley (1972) showed that acute treatment with the antidepressant imipramine (10 and 20 mg Kg^{-1}) reduced shock-induced fighting, while Sheard et al. (1977) demonstrated that 5 mg Kg^{-1} of either clomipramine or

desipramine reduced the LSD-induced potentiation of shock-induced aggressive behaviour at doses which did not affect shock-induced aggression per se. The effects of tricyclic antidepressants on shock-induced aggression therefore appear to be somewhat equivocal.

The hyper-aggressive behaviour of mice following long-term isolation may be reduced specifically (i.e. at doses which show no neurotoxicity or locomotor impairment) by numerous pharmacological classes of compounds administered acutely (DaVanzo et al., 1966; Malick, 1979), e.g. neuroleptics, antidepressants, anticholinergics, antihistaminics (effects of which may well be linked to associated anticholinergic activity) and drugs which either deplete 5-HT (i.e. pCPA) or are 5-HT antagonists (e.g. mianserin, methiothepin or methysergide). Conversely anxiolytics, sedatives or hypnotics, narcotic analgesics, muscle relaxants and anticonvulsants reduced aggression induced by long term isolation non-selectively (see also Valzelli et al., 1967). The ability of acute treatment with pCPA to reduce aggressive behaviour in long term isolated mice (which is supported by Rolinski, 1975a) is surprising considering that similar treatment in non-isolated rodents induces aggressive behaviour. Similarly, aMpT also reduced aggressive behaviour in long term isolated mice (Rolinski, 1975a) but induced muricide in rats (McLain et al., 1974). These diametrically different results are probably due to either species differences, the treatment regimes used or the biochemical changes in mice observed following long term isolation (Valzelli, 1978). As noted previously, long term isolation of rats (10-12 months) results in a complex behavioural syndrome where locomotor activity is elevated compared to non-isolated controls (Garzon et al., 1979). In

isolated rats TCA's (amitriptyline, clomipramine, desipramine and doxepin), atypical antidepressants (viloxazine and trazodone) and MAOI's (phenelzine and clorgyline) all reduced the elevated locomotor activity at doses which did not modify rotarod performance (Garzon et al., 1979). Conversely, in the same studies, neuroleptics (chlorpromazine and haloperidol) and anxiolytics (chlordiazepoxide and diazepam) had no effect at non-sedative doses.

Table 8.1 compares the relative potencies of the drugs examined in the current series of experiments to those on other models of aggressive behaviour quoted (where available) from the literature cited above. The table indicates that clomipramine and diazepam exhibit near-equal potencies on the various forms of aggressive behaviour. Fluoxetine, iprindole, mianserin (its potency on isolation-induced aggressive behaviour in mice excepted), phenelzine and haloperidol, however, are all far more potent (between 1 and 2 orders of magnitude) on aggressive behaviour exhibited by resident animals during social interaction with an unknown conspecific intruder than on the other animal models of rodent aggressive behaviour. With only two exceptions, the cited studies administered each drug intraperitoneally compared to the subcutaneous route used in the current studies. However, it is not thought that the respective pharmacokinetic properties would explain the large differences in potency observed for each drug.

Drug	Current SI studies	Spontaneous Killer-rats Muricide	Electro-shock induced aggression	Isolation induced (mice)
Clomipramine	9.32 sc	10 c	>10 c	7.2 po f
Fluoxetine	0.93 sc	3.3-30 d (>10.0* a)		
Iprindole	1.59 sc			12.5 f 32.5 po f
Mianserin	1.05 sc	10 c	>10 c	0.5 f
Phenelzine	0.77 sc	5 e		33.4 b
Haloperidol	0.07 sc	>2.5 c		1 c
Diazepam	2.7 sc	5 c	3-5 c	6.7 b

Table 8.1 Comparison of the ID_{50} values of antidepressant drugs, haloperidol and diazepam on rodent aggressive behaviour.

ID_{50} values; mg Kg^{-1} ip (unless route specified)

Species; rat (unless specified)

SI; social interaction studies (mg Kg^{-1} values were calculated from the umol Kg^{-1} values presented in the text)

*; pCPA-induced muricide

References cited. a, Berzsenyi et al. (1983); b, DaVanzo et al. (1966); c, Delini-Stula and Vassout (1979); d, Gibbons et al. (1978b); e, Horovitz et al. (1966); f, Malick (1979) and references cited therein

The results of the present study generally confirm those of other workers that acute treatment with antidepressants specifically reduced aggressive behaviour in rats without inducing motor impairment. It should be noted, however, that this study has demonstrated such drug-induced effects without resorting to the extreme methods of inducing aggressive behaviour such as pCPA-, aMpT- or olfactory bulb lesion-induced muricide, shock-induced aggression or aggression resulting from long-term isolation. In addition, the social interaction test as described in this thesis appears to be an extremely sensitive measure of the effects of acute psychotropic drug treatment on endogenous patterns of rodent social behaviour and especially sensitive to the anti-aggressive properties of psychotropic compounds following acute treatment than other animal models of aggression.

Whether the antidepressant-induced reduction in aggressive behaviour is a specific effect only exhibited by the antidepressants is open to question since similar drug-profiles have been obtained for examples of amphetamine-like compounds, anticholinergics and antihistaminics (Horovitz et al., 1966; Malick, 1976). The anti-aggressive properties of psychotropic compounds have often been used as an argument for their potential anxiolytic activity, e.g. McMillen et al. (1987) extolled the anxiolytic potential of gepirone after identifying its anti-aggressive properties on the behaviour of long-term isolated mice. If this argument is valid then the specific reduction in aggressive behaviour observed following acute antidepressant treatment in these studies would be indicative of anxiolytic activity. As Rodgers and Waters (1985) have argued, and in consideration of the effects of numerous classes of psychotropic

drugs on the different forms of aggressive behaviour expressed by rodents outlined above, to employ the drug-induced inhibition of aggressive behaviour per se as an argument for anxiolytic activity is invalid. No claim is therefore made that the specific effect of on rodent social behaviour observed in these studies following acute antidepressant treatment is indicative of anxiolytic activity.

It is widely accepted that in the clinic at least 2-3 weeks of continuous antidepressant treatment is required before any beneficial effects may be observed (Oswald et al., 1972). It is thus remarkable that pharmacologists/biochemists in general have largely ignored this fact and have been quite content to theorize on the mode of action of antidepressants on the basis of animal studies utilizing acute drug-treatment regimes. Very few extensive studies on the effect of chronic antidepressant treatment on rodent social behaviour (in its many guises) have therefore been published. Even so, a surprisingly clear picture emerges given the paucity of published data (see by Vogel et al., 1986).

Valdman and Poshivalov (1986) demonstrated that chronic (7 day) treatment of mice with fluoxetine and iproniazid (both at 10 mg Kg^{-1} day^{-1}) largely restored intraspecies social behaviour during social interaction following behavioural depression induced by reserpine. In the same studies, similar treatment regimes with trazodone, pyrazidol and clomipramine (but not zimelidine) reversed both the suppression of aggressive behaviour and the increased defensive behaviour of dominant mice induced by nociceptive electrical stimulation given repeatedly over 14 days. Likewise, the social interaction experiments of Willner et al. (1981) showed that rats

treated chronically (7 day) with desipramine (20 mg Kg^{-1} day^{-1}) were more likely than controls to attack a conspecific intruder.

Elevated aggressive behaviour has also been observed in the shock-induced fighting model following chronic treatment of rats with antidepressants. Prasad and Sheard (1982) showed that rats treated with desipramine (15 mg Kg^{-1} day^{-1}) exhibited increased fighting after 14 days of treatment. Conversely, lithium reduced fighting behaviour when administered over 14 days via the drinking water (20 mEq L^{-1}). Similarly, Mogilnicka and Przewlocka (1981) demonstrated increased shock-induced fighting in rats that had received 10 days treatment with 10 mg Kg^{-1} day^{-1} (given twice daily) of amitriptyline, imipramine, iprindole or mianserin. Increased shock-induced aggressive behaviour was also observed by Eichelman and Barchas (1975) following 3-5 days of treatment with imipramine, amitriptyline and desipramine (10 mg Kg^{-1} day^{-1}) and from 30 hours following the start of treatment with the MAOI's iproniazid (150 mg Kg^{-1} day^{-1}), nialamide (100 mg Kg^{-1} day^{-1}) and pargyline (20 mg Kg^{-1} day^{-1}). Although it has previously been argued that shock-induced aggressive behaviour of rats by electro-shock is indicative of defensive behaviour this should not be taken to indicate increased submission, rather, such behaviour is indicative of aggressive activity but in the defensive context. It should be noted here that during social interaction rats treated chronically with desipramine, although more likely to attack the conspecific intruder, were also more likely to submit when attacked (Willner et al., 1981). At odds with these observations is the report by Cairncross et al. (1979) which indicated that the hyperactivity and hyper-reactivity induced by olfactory bulbectomy were reversed (i.e. animals became

normalised) following chronic (10-14 day) treatment with amitriptyline, iprindole, mianserin, nomifensine and viloxazine but not with the MAOI tranylcypramine. It should also be noted, however, that sham-operated rats exhibited higher irritability scores following chronic treatment with mianserin (5 mg Kg^{-1} day^{-1}) than those receiving saline control.

Maj and co-workers have extensively studied the effect of acute and chronic psychotropic drug treatment on the aggressive behaviour of mice and rats induced by acute treatment with clonidine (alpha-adrenoceptor agonist) or apomorphine (DA agonist) respectively. In their mouse studies 4 mice (from the same home cage) were placed in a glass cylinder immediately following treatment with clonidine, 20 mg Kg^{-1}, and the number of biting attacks observed over 1 hour recorded. In the rat studies 2 rats (from the same home cage) were paired in wire cages 10 min. after treatment with apomorphine, 5 mg Kg^{-1}, and the number of fighting pairs recorded. Fighting behaviour in rats was defined when both rats assumed a mutual upright (boxing) posture, identical to that observed during shock-induced aggression, or when one rat forced its partner to adopt a submissive posture. These studies (Maj, 1984; Maj et al., 1979; 1980; 1981; 1982) showed that chronic (14 day) treatment with TCA's, MAOI's and atypical antidepressants invariably increased clonidine- and/or apomorphine-induced aggressive behaviour at doses that either reduced drug-induced aggressive behaviour or had no effect when given acutely. The only exceptions were observed in the mouse experiments with antidepressants which reportedly exhibit 5-HT specificity, i.e. fluoxetine, which decreased clonidine-induced aggressive behaviour following acute and chronic

treatment; citalopram, which reduced aggressive behaviour after acute administration but had no effect when given chronically; and fluvoxamine, which had no effect following acute or chronic administration. Interestingly, the serotonin antagonist, pizotifen, and the neuroleptics, thioridazine and trans-flupenthixol, also increased clonidine-induced aggressive behaviour when given chronically; this effect was thought by Maj and co-workers to be due to the noradrenolytic activity possessed by these compounds (Maj et al., 1982). Clonidine-induced aggressive behaviour in mice is thought to be mediated via alpha$_1$-adrenoceptors (sic) and thus the potentiation of such behaviour observed following chronic antidepressant treatment was argued to be due to enhanced central noradrenergic activity (Maj, 1984). These workers also observed that the potentiation of apomorphine-induced fighting behaviour observed following chronic amitriptyline treatment could be blocked by acute treatment with either phenoxybenzamine (alpha-adrenoceptor antagonist) or spiperone (DA antagonist) but not by metergoline (5-HT$_2$ antagonist). In addition, acute treatment with phenoxybenzamine potentiated, while spiperone blocked, the apomorphine-induced stereotypy exhibited by rats treated chronically with amitriptyline. On the basis of the antagonist activity of phenoxybenzamine it was argued that the potentiation of apomorphine-induced fighting by chronic antidepressant treatment was due to increased noradrenergic transmission (Maj et al., 1979). The mechanism(s) involved in the mediation of apomorphine-induced aggression, however, are far from clear. For example, small doses of clonidine (0.125 mg Kg^{-1}) are sufficient to potentiate apomorphine-induced aggression (Gianutsos et al., 1976). Furthermore, both morphine and the neuroleptics haloperidol and

oxyperomide demonstrably decrease such behaviour and, while the effect of morphine is sensitive to naloxone, the anti-aggressive properties of oxyperomide may be blocked by the cholinergic antagonist dexetimide or potentiated by the muscarinic agonist pilocarpine (Gianutsos and Lal, 1976). The initial impressions from the work of Maj and co-workers are encouraging since these workers appear to have identified a degree of commonality between the chemically-disparate group of compounds labelled antidepressant, with the exception of those compounds with serotonergic specificity (i.e. fluoxetine, fluvoxamine and citalopram). These animal models of aggression may well prove to be invaluable in identifying potential antidepressants whose effects are mediated via changes in noradrenergic, but not serotonergic, transmission. Unfortunately, however, the work of Maj and co-workers is open to criticism. None of the published experiments quoted above appear to be adequately controlled since all these reports failed to indicate the effect of chronic psychotropic drug treatment per se on the aggressive behaviour of mice or rats using their experimental methods. Conversely, the present study has demonstrated that chronic antidepressant treatment per se promotes aggressive behaviour in rats. The apparent potentiation of clonidine- or apomorphine-induced aggressive behaviour identified by Maj and co-workers following chronic antidepressant treatment may therefore simply be due to an additive effect of the two treatment regimes on rodent behaviour induced by separate mechanisms.

All of the chronic drug-treatment studies cited above employed one or more of the antidepressants examined in the current series of experiments. In each case, however, the drug in question was

administered via bolus intraperitoneal injection at 10 mg Kg^{-1} either daily over 7 days (clomipramine and fluoxetine; Valdman and Poshivalov, 1986) or 10 days (iprindole and mianserin; Mogilnicka and Przewlocka, 1981; clomipramine, iprindole and mianserin; Maj et al., 1979) or bi-daily (iprindole and mianserin; Maj et al., 1980; fluoxetine, Maj et al., 1981). No comparable data is available for phenelzine. In the social interaction experiments reported here, however, resident rats received mini-osmotic pumps implanted subcutaneously in order to administer target doses (defined as the minimally-effective dose identified when given acutely) continuously over 24 hours, i.e. clomipramine (3.14 mg Kg^{-1} day^{-1}), fluoxetine (0.34), iprindole (0.86) and mianserin (0.087); the mg Kg^{-1} values were calculated from the umol Kg^{-1} values reported in the text. With the exception of clomipramine, the antidepressants examined demonstrably increased the aggressive behaviour of resident rats at doses between 1-2 orders of magnitude lower than those employed in the cited literature. The social interaction test therefore appears to be a more sensitive test of the effects of chronic antidepressant treatment on rodent behaviour than those animal models cited above.

The results of the present study generally confirm those of other workers that chronic treatment with the chemically-disparate antidepressants specifically increases aggressive behaviour in rats. As with the acute drug-treatment social interaction studies, however, such drug-induced changes in rodent behaviour were observed without resorting to extreme models of inducing (or reducing) aggressive behaviour. In addition, the level of aggression exhibited by resident rats during social interaction appears to be an

extremely sensitive measure of the ability of chronic antidepressant treatment to increase social drive. Indeed, as discussed in the review by File and Tucker (1986), aggression is the only type of rodent social behaviour consistently increased following chronic treatment with antidepressants; no clear generalisation of effect has been observed in studies examining the effect of chronic antidepressant treatment regimes on drug-discrimination, measures of reward and punishment (intracranial self-stimulation or intake of food and water), exploratory locomotion or the various measures of the response to acute or chronic stress.

As previously discussed, aggression in rodents may be induced by reducing central 5-HT and/or noradrenergic activity or increasing dopaminergic activity, or, conversely, reduced by increasing central 5-HT and/or noradrenergic function or decreasing dopaminergic activity. By implication therefore, the inhibitory effects of acute antidepressant treatment on rodent aggression may be associated with an ability to increase serotonergic and/or noradrenergic function which (with the exception of iprindole, where the acute mode of action has yet to be elucidated) is in accordance with the accepted modes of action of the majority of antidepressants, i.e. inhibition of transmitter re-uptake or MAO activity, or blockade of pre-synaptic alpha$_2$-adrenoceptors (mianserin) all of which result in increased synaptic concentration of the neurotransmitter. In addition, the argument also implies that increased aggressive behaviour induced by chronic antidepressant treatment is associated with decreased serotonergic and/or noradrenergic function or increased dopaminergic activity. Before discussing the implications of such findings to the commonly-accepted theories on the aetiology of depression it is

pertinent at this juncture to attempt to relate the observed behavioural changes induced by acute and chronic antidepressant treatment in rodents to the clinical efficacy of such compounds used in the treatment of affective disorders.

Prior to successful clinical treatment depressed patients are unable to cope with the everyday stresses of normal life but as the treatment progresses so the individual's responses to external environmental and social stimuli change. It has long been thought by psychiatrists that at least part of the symptomology of depression is manifest as inwardly-directed aggression, i.e. aggression directed against oneself, the ultimate expression of which is suicide. One measure which attempts to quantify the relative levels of inwardly- and outwardly-directed aggression is the Hostility and Direction of Hostility Questionnaire (HDHQ; see Priest et al., 1980, and references cited therein). Data arising from the HDHQ is grouped into 2 intropunitive scales (i.e. self-criticism and guilt) and 3 extrapunitive scales (i.e. urge to act out hostility, criticism of others and projected hostility). Depressive illness is associated with relatively high levels on the intropunitive scales compared to those on the extrapunitive scales. A reduction in the intropunitive aspects of depression (such that the scores become biased towards the extrapunitive scales) is associated with remission from the illness. Kaplan et al. (1961) took the argument one stage further. They suggest that increased outwardly-directed aggression, manifested as increased physical and/or verbal activity associated with greater interaction with the environment and usually of a constructive nature, is an integral component of the recovery process from depression. Such changes in the patient's response to external

stimuli only occur during chronic antidepressant treatment and are not normally observed until 10-20 days of drug treatment have elapsed (Oswald et al., 1972). Although the argument involves a degree of anthropomorphism the parallel between the observations of Kaplan and Priest and their respective co-workers to the data presented in this report is clear; chronic treatment with antidepressants increase certain manifestations of aggressive behaviour in both rodents and depressed human patients. If the parallel to the clinical situation were complete then would not acute treatment with antidepressants (i.e. the initial doses of clinical treatment), which in rodents decreases aggressive behaviour, exacerbate the depressive symptomology ?, and is there any evidence of such acute effects in the clinic ? Oswald et al. (1972) suggest that indeed this is the case. 12 normal male and female young adults indicated that during the first week of imipramine, 75 mg, administration they felt depressed in mood. By the third week of administration, however, their self-rated mood had returned to normal. In comparison, depressed patients frequently complain of feeling worse during the initial stages of drug treatment, but such complaints are either ignored, and categorised as side-effects (Oswald et al., 1972), or simply not registered because of the inadequacies of the clinical rating system of depression used. The Hamilton Rating Scale (HRS; Hamilton, 1960) has been the most widely used method of assessing the severity of depression since its inception. Ratings are based on clinical interview which aims to cover the preceding week and 21 items, including both mental and physical symptoms, are scored and the values summed to produce an overall rating of the severity of depression. Two major criticisms may be levelled at this method. Firstly, ratings cannot be made more frequently than at weekly

intervals and, secondly, since the overall rating is a summation of scores, opposing movements of individual scores are hidden. Thus any increase in depressed mood, guilt or suicidal tendancy (indicative of increased depressive symptomology) may be effectively masked by reduced intensity of the somatic symptoms and increased drive. No net change in the HRS score is therefore seen until the majority of items show improvement and this probably accounts for the apparent lack of effect experienced during the initial stages of antidepressant therapy. A rating scale such as the HRS is therefore incapable of identifying specific changes in the individual items associated with the symptomology of depression especially during the initial stage of treatment. It is now accepted by some psychiatrists, however, that a potentially hazardous situation may arise during the initial stages of antidepressant treatment where the drive of a depressed patient is restored while the feelings of pessimism and hopelessness remain (Priest et al., 1980). With the resurgence of activity a suicide attempt becomes a possibility especially during the early stages of treatment.

It may be argued, therefore, that the drug-induced changes in rodent social behaviour following both acute and chronic antidepressant treatment correspond quite closely to the time-course of antidepressant effects observed in the clinic. In addition, it has been argued (see above) that the increase in social drive, which in rats at least is manifested as increased aggressive behaviour, is associated with reduced serotonergic and possibly noradrenergic activity or increased dopaminergic activity in the brain. The argument therefore implies that prior to antidepressant therapy central serotonergic/noradrenergic activity is abnormally high,

while that of the central dopaminergic systems may be relatively reduced. If the argument is limited to the central pre-synaptic effects of antidepressants then it appears to oppose the classical theories on the monoamine hypothesis of depression postulated by Coppen (1967) and Schildkraut and Kety (1967) which, based on the ability of tricyclic antidepressants and MAOI's to increase the synaptic concentration of central neurotransmitters, state that depression may be due to underactive serotonergic/noradrenergic systems (see section 1.6.2). The classical theories of depression, however, are based solely on pre-synaptic events and ignore both the latency of clinical effect experienced with these drugs and the plasticity of central monoaminergic systems. It must be assumed that administration of antidepressants over a long period of time modifies the relative balance of events involved in monoaminergic neurotransmission, thereby altering the net level of activity of the neuronal network, which, at the synaptic level, is dictated by the post-synaptic receptiveness and sensitivity of the second messenger systems (e.g. adenylate cyclase and phosphoinositidase C activity) regardless of the level of transmitter release. It has been demonstrated that chronic, but not acute, treatment with tricyclic antidepressants leads to decreased accumulation of cAMP stimulated by exogenous NA in rat cortical (Frazer and Mendels, 1977) and limbic (Vetulani et al., 1976a) structures. These observations indicate either reduced sensitivity of receptors linked to the stimulatory G-protein of the adenylate cyclase second messenger system, i.e. beta-adrenoceptors (Lefkowitz and Hoffman, 1980; Strosberg et al., 1982), or increased sensitivity of receptors linked to the inhibitory G-protein of the same system, e.g. $alpha_2$-adrenoceptors (Exton, 1982) or $5\text{-}HT_{1A/1B/1D}$ receptors (Conn and Sanders-Bush,

1987; Fozard, 1987). Down-regulation of central beta-adrenoceptors alone would therefore account for the reduced accumulation of cAMP stimulated by exogenous NA, and indeed reduced beta-adrenoceptor number have been observed following chronic treatment with amitriptyline, desipramine, doxepin, imipramine, iprindole, mianserin and pargyline (Banerjee et al., 1977; Mishra et al., 1980; Peroutka and Snyder, 1980). In addition, Crews and Smith (1978) showed that chronic treatment with desipramine decreased the sensitivity of pre-synaptic alpha$_2$-adrenoceptors which mediate the negative feedback system regulating the release of further transmitter. Thus down-regulation of pre-synaptic alpha$_2$-adrenoceptors or continual blockade of these receptors by, for example, mianserin would lead to the increased outflow and hence synaptic concentration of NA resulting in post-synaptic beta-adrenoceptor subsensitivity.

Likewise post-synaptic 5-HT$_2$ receptors, linked to the stimulatory G-protein of phosphoinositidase C system (Minchin, 1977; Fozard, 1987), are also down-regulated by chronic treatment with amitriptyline, desipramine, imipramine, iprindole and pargyline (Peroutka and Snyder, 1980), probably in response to the increased synaptic concentration of serotonin.

For both central noradrenergic and serotonergic systems, therefore, there is evidence of down-regulation following chronic antidepressant treatment, implying that in depression the second messenger systems may be relatively over-sensitive to endogenous NA or 5-HT. The antidepressant-induced beta-adrenoceptor and/or 5-HT$_2$ receptor subsensitivity therefore indicate a return to normality. This latter

hypothesis on the underlying causes of affective disorders and the mode of action by which antidepressants produce amelioration of depression provides the basis of the alternative monoamine hypothesis first forwarded by Vetulani, Sulser and colleagues (Vetulani et al., 1976a; Sulser et al., 1978; see also section 1.6.2). The relative merits of both the classical and alternative hypotheses have been the topic of major discussion in the literature (e.g. van Praag, 1981a) and the overriding impression is that the two hypotheses have each been treated as mutually exclusive. In fact both hypotheses may be mutually complementary. If the second messenger systems in depression are indeed over-sensitive to the synaptic transmitter levels then negative feedback systems would come into play to reduce further transmitter release. To re-dress the balance post-synaptic receptor stimulation must first be increased (by, for example, increasing the synaptic concentration of the endogenous neurotransmitter, thus giving rise to the classical hypothesis) in order to induce down-regulation of the post-synaptic receptors, thereby reducing the sensitivity of the second messenger systems (i.e. alternative hypothesis) to a level lower than that prior to antidepressant treatment.

CHAPTER 9 CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN RATS

CHAPTER 9 CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN RATS

9.1 Introduction

The clinical evidence implicating circadian rhythm abnormalities in affective disorders, which led to the suggestion by some workers that certain forms of depression may be produced by a disruption or de-synchronization of circadian rhythms (Wehr and Wirz-Justice, 1982; Wehr et al., 1982; Kripke, 1983; Wehr, 1988), have already been described (see section 1.6.3). These observations led Wehr and Wirz-Justice (1982) to propose that if phase-advances of circadian rhythms were a primary pathophysiological mechanism of some depressive states then the clinical efficacy of antidepressants may depend on an ability to induce a phase-delay in those circadian systems abnormally advanced.

The presence of a circadian rhythm in the locomotor activity of rodents is well established and has been shown to be modified by some antidepressant drugs. Thus, clorgyline, imipramine and lithium carbonate have each been demonstrated to lengthen the free-running activity rhythm of hamsters (Wirz-Justice and Campbell, 1982; Wirz-Justice, 1983, and references cited therein) or blinded rats (Kripke and Wyborney, 1980). In addition, imipramine promoted the dissociation of the activity rhythms (Wirz-Justice and Campbell, 1982). Whether the ability to induce a phase-delay or dissociation in certain circadian rhythms is an ability shared by the group of compounds labelled antidepressant has not been established. The purpose of the following experiments was to determine whether the antidepressants clomipramine, fluoxetine and mianserin shared the ability to modify circadian rhythmicity by examining the effect of chronic treatment with these compounds on the free-running circadian

locomotor activity rhythm of rats.

9.2 Materials and Methods

9.2.1 Subjects

Male Wistar rats, 175-200g at the start of each experiment, were taken from stock and housed either singly or in groups of three in the respective locomotor activity monitors (see section 4.3 and 4.4). In the case of the grouped experiments the animals within each group were weight-matched.

9.2.2 Experimental Design

When monitoring the overall circadian locomotor activity of animals maintained in isolation or in groups it is important to use a reliable, robust system that provides an accurate measure of activity, together with control over the environmental conditions, while still allowing the animals to exhibit their normal behaviour patterns. Various behavioural parameters have been used to study circadian activity in rodents (e.g. locomotor activity, feeding and drinking behaviour) the most common of which appears to be locomotor activity. Different tools have been used to measure circadian locomotor activity (e.g. running wheels, horizontal activity electromagnetic sensors) but such systems may influence the results of animal experiments for free-running rhythms (Yamada et al., 1986). The use of infrared photocells to obtain an index of locomotor activity in both acute and long-term studies is becoming more common and such a system has been used in the following studies. Infrared photocell systems have advantages over other locomotor activity monitors in that they do not require excessive exercise as

with running wheels, nor are they so sensitive as to record periods of activity not associated with locomotion, such as a change of resting body posture or grooming activity, which may be a problem with sensitive electromagnetic sensors. For a description of the infrared photocell systems used in these studies together with the practical considerations required when using such systems see section 4.4.2.

9.2.2.1 Environmental Cabinets

All experiments described in this chapter were performed with the subjects housed in environmental cabinets, described in section 4.3., containing the relevant locomotor activity monitor (section 4.4.2).

9.2.2.2 Measurement of Locomotor Activity

Details of the programmes used to capture, store and analyse circadian locomotor data have been presented by Marshall et al. (1985); see also section 4.4.2.4. In all of the following experiments activity scores were aggregated every 15 min throughout the duration of each experiment and stored on floppy-disk for analysis at a later date.

9.2.2.3 Drug Treatment

Drugs were administered orally via the drinking water. Where animals were housed individually, water consumption was measured every 2-3 days, taking care not to disturb the subjects. In the case of grouped animals, water consumption was usually measured daily since the reservoir was positioned externally to the environmental cabinet. In all cases the concentration of the drug solution provided was

modified according to the daily volume consumed since the previous measurement together with the assumed weight gain of the subjects during the duration of each experiment (obtained from previously determined growth-curves) in order to approach as closely as possible the desired dosage. In the grouped experiments the drug concentrations were calculated on the basis of the assumed total weight of the group with the further assumption that drinking volume was evenly distributed between the subjects. The daily target dosage of each test drug used in these studies (i.e. clomipramine, 20 mg Kg^{-1} base; fluoxetine, 2 and 6 mg Kg^{-1} pfb and mianserin, 2 mg Kg^{-1} base) was based on previous data for clomipramine (Martin, 1982), the relative potency on rodent social behaviour (see sections 5.5.2 and 5.5.3), and estimates of clinical equivalence (see section 3.3). Clomipramine and mianserin were administered for at least 20 days while fluoxetine was administered for at least 30 days. All drugs were presented as described in section 4.6. Prior to and following drug treatment all animals received polished water (as defined in section 4.6) as the standard drinking solution. In control studies animals received polished water throughout the duration of the experiment. The effect of chronic treatment with each drug was examined on at least two occasions in both isolated and grouped animals.

9.2.2.4 Duration of Experiments

As discussed previously (sections 3.2 and 9.2.2.3) the duration of each experiment was determined not only by the duration of drug treatment but also by the data requirements of the Time-Series analysis employed (see also section 9.2.3).

Thus each experiment was divided into blocks of data (each of at least 10 days in duration) as follows;

Data Block	Lighting Conditions	Drug Treatment
1	L:D	None
2	D:D	None (Pre-dose)
3	D:D	Drug (clo, mian, fluox)
4	D:D	Drug (clo, mian, fluox)
5	D:D	None (Post-dose; clo, mian) Drug (fluox)
6	D:D	None (Post-dose; fluox)

L:D; Normal lighting (12h/12h, lights on 0700 or 0800)

D:D; Constant dark (isolated animals) or constant low-intensity red light (6 lux; grouped animals).

clo, clomipramine; mian, mianserin; fluox, fluoxetine.

In some experiments the initial 10 day period of L:D was omitted. Generally, both isolated and grouped animals were allowed a few days acclimatization to the respective activity monitor under L:D conditions. Following the post-drug monitoring period all animals were maintained under L:D or D:L (i.e. reverse-lighting conditions) prior to the termination of the experiment.

9.2.3 Data Analysis

Circadian locomotor activity experiments produce a plethora of data which must be collated and analysed. In addition some means of visual representation of the data must be produced. In these studies the data has been presented graphically by means of actograms (section 9.2.3.1) or by subjecting the data to Time-Series (periodogram) analysis to identify the periods of rhythmicity of locomotor activity (section 9.2.3.2). For brevity, the graphical

representations of the data or spectral analysis will only be provided to illustrate particular results.

9.2.3.1 Actograms

An actogram is a visual display of the data activity distribution over 24h, double-plotted to facilitate visual inspection. Each bar in the actogram represents a sampling interval where the number of locomotor activity counts are equal to or higher than the predetermined threshold. Actograms using a constant threshold value produce a high-pass filter effect on the data. This may be perfectly satisfactory where the distribution of data is constant from day to day. The use of actograms employing a constant threshold on data where the daily distribution of the data changes is open to criticism, however, since the threshold may be set in order that the actogram produces the impression of circadian activity required by the experimenter. A far more acceptable method of producing actograms is to allow the data to determine the threshold level on which the actogram is plotted. This may be achieved by determining either the mean or semi-quartile values of the activity distribution calculated over a predetermined period (e.g daily) of the experiment. Circadian locomotor activity data is highly skewed (see appendix B.4) and for this reason the use of the mean of daily activity as the threshold value is unacceptable. The actograms presented in these studies employed the calculated daily median of locomotor activity as the threshold of activity from which the actogram was plotted.

9.2.3.2 Time-Series analysis

A time series is a collection of observations made sequentially in time. In these studies the applicability of two methods of

time-series analysis, i.e. periodogram analysis and autocovariance function analysis, have been applied to circadian locomotor activity data. A simple derivation of the relevant equations is given in appendix B, together with a short discussion on the merits of each method.

Autocovariance function analysis assumes that the data is normally distributed (i.e. equally distributed about the sample mean). Circadian locomotor activity data arising from both individual and grouped animals are highly skewed however, and thus the raw data obtained in these studies must be transformed in order to normalize, as far as possible, its distribution (see appendix B.4). Only then may this method of time-series analysis be applied. Cube-root transformation invariably normalizes the distribution of the data arising from grouped animals but also results in a drastic reduction in the magnitude of the locomotor activity peaks. The resulting flattening of the activity profile reduces the ability of this method to identify significant periods of rhythmicity. In addition, in the studies described here the magnitude of the data arising from individual animal experiments is poor such that the majority of epochs contain zero measures of locomotor activity; root transformation of the data does not result in normalizing the distribution and is thus not applicable. The use of autocovariance function analysis to either raw locomotor activity data or following cube-root transformation is therefore highly questionable. Conversely, periodogram analysis makes no assumption on the distribution of the data and thus may be applied to the raw data. For this reason only the results of periodogram analysis will be presented.

9.3 Results

9.3.1 Polished H_2O

9.3.1.1 Individual animals

During L:D conditions all isolated rats exhibited an entrained circadian rhythm of locomotor activity with a fundamental period of between 23.75h and 24.75h as determined by periodogram analysis (Table 9.1). Visual inspection of the actograms (an example of which is given in Fig. 9.1 together with the derived periodograms in Fig. 9.2) suggests that on exposure to D:D all animals exhibited a free-running circadian rhythm of locomotor activity with a fundamental period slightly greater than 24h., which was maintained throughout the experiment until the re-introduction of the 12h./12h. light-dark cycle. Periodogram analysis of sequential 10 day periods during D:D indicates the fundamental period of circadian locomotor activity to be 24±0.25h. (Table 9.1) and the circadian rhythm of locomotor activity exhibited during D:D to be essentially consistent (see Fig. 9.2) with, in the majority of cases, no evidence of rhythm disruption.

9.3.1.2 Grouped animals

Grouped animals exhibited an entrained circadian rhythm of locomotor activity during L:D (12h. lights on/12h. lights off) with a fundamental period of 24h. (Table 9.2) as determined by periodogram analysis. During D:D grouped animals exhibited a free-running rhythm with an initial fundamental period of 24.5h., which gradually increased during each experiment. No spontaneous breakdown in the circadian rhythm (i.e. disruption) was observed. An example actogram together with the derived periodograms are provided in Figs. 9.3 and 9.4 respectively.

Animal	Lighting Conditions (days)	Predominant Periods (h)	
1	L:D (1-10)		24.25
	D:D (13-22)	(8.00, 11.25, 13.00) (18.25, 28.25)	24.25
	D:D (23-32)	(6.00)	24.00
	D:D (33-42)	(4.75, 6.00)	24.25
	D:D (43-52)		24.00
2	L:D (1-8)	(8.00,12.25)	24.50
	D:D (13-22)	(8.00, 12.00, 28.00)	24.00
	D:D (23-32)	(8.00, 12.25)	24.00
	D:D (33-42)	(8.00, 12.00)	24.25
	D:D (43-52)	(6.00, 8.00, 12.00)	24.25
3	L:D (1-10)	(1.25, 4.00, 4.75) (6.00, 8.00, 8.75) (12.25, 13.75)	23.75
	D:D (13-22)	(8.00, 12.00)	23.75
	D:D (23-32)	(8.00)	24.00
	D:D (33-42)	(3.00)	24.25
	D:D (43-52)		24.00
4	L:D (1-10)	(8.25, 12.00)	24.75
	D:D (13-22)	(8.00, 20.50, 27.00)	23.50
	D:D (23-32)	(8.00)	24.00
	D:D (33-42)	(6.25, 8.00, 9.00) (12.75, 19.25, 21.25) (27.50)	24.25
	D:D (43-52)	(6.00, 8.00, 12.00)	24.25

Table 9.1 Periodogram analysis of the circadian rhythm of locomotor activity expressed by untreated individual rats.

L:D, normal lighting (12h light/12h dark) days 1-12; D:D, constant darkness from day 13; values in parenthesis indicate days used for spectral analysis

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for animal 2 are shown in Figs 9.1 and 9.2 respectively.

Figure 9.1 Actogram of the circadian rhythm of locomotor activity expressed by an untreated isolated rat.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Normal lighting (12h light/12h dark, lights on 0800), days 1-12.
Constant darkness, days 13-53.
Normal lighting (as above), days 54-60.

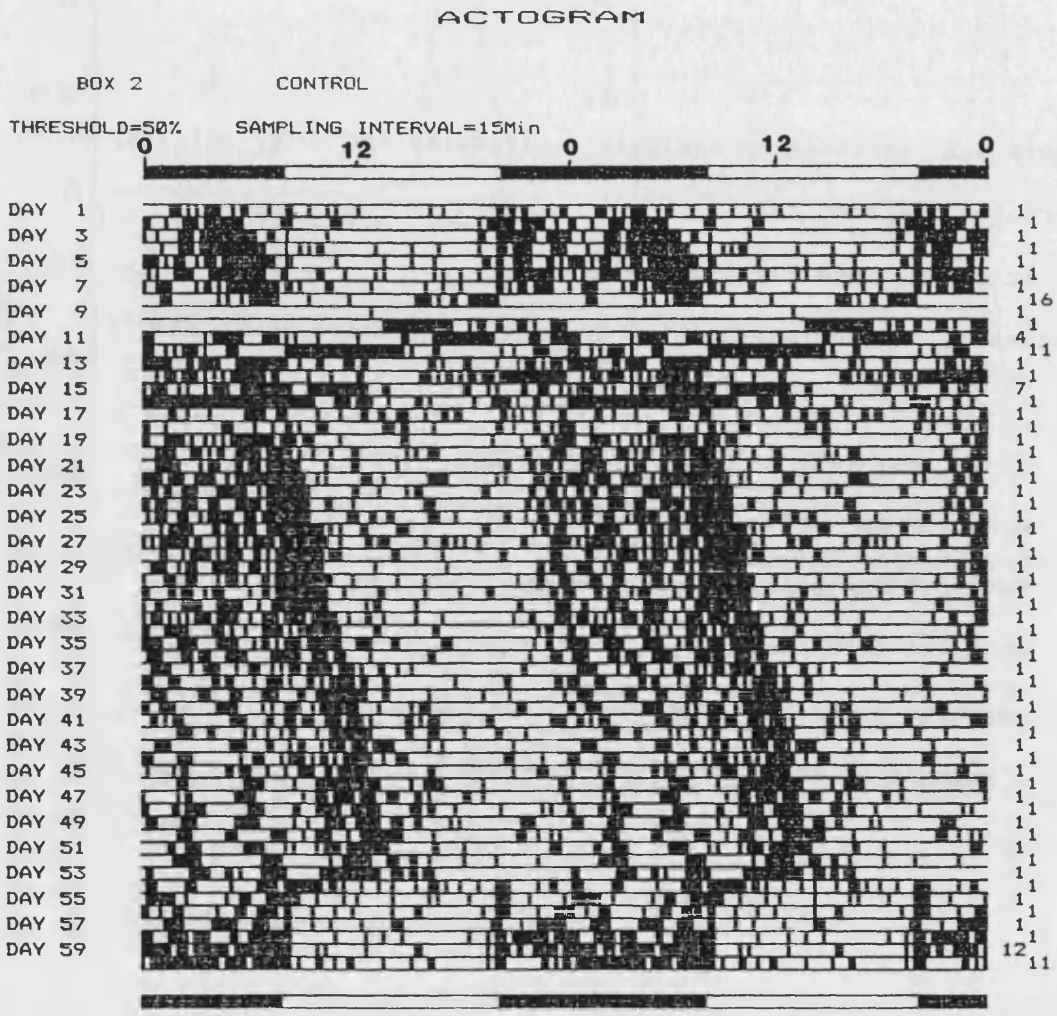

ACTOGRAM
BOX 2 CONTROL
THRESHOLD=50% SAMPLING INTERVAL=15Min
0 12 0 12 0
DAY 1
DAY 3
DAY 5
DAY 7
DAY 9
DAY 11
DAY 13
DAY 15
DAY 17
DAY 19
DAY 21
DAY 23
DAY 25
DAY 27
DAY 29
DAY 31
DAY 33
DAY 35
DAY 37
DAY 39
DAY 41
DAY 43
DAY 45
DAY 47
DAY 49
DAY 51
DAY 53
DAY 55
DAY 57
DAY 59

Figure 9.2 Periodogram analysis : Circadian locomotor activity of an untreated isolated rat.

For actogram, see Fig. 9.1.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{4}$).

a) Normal daylight (12h light/12h dark), days 1-8.

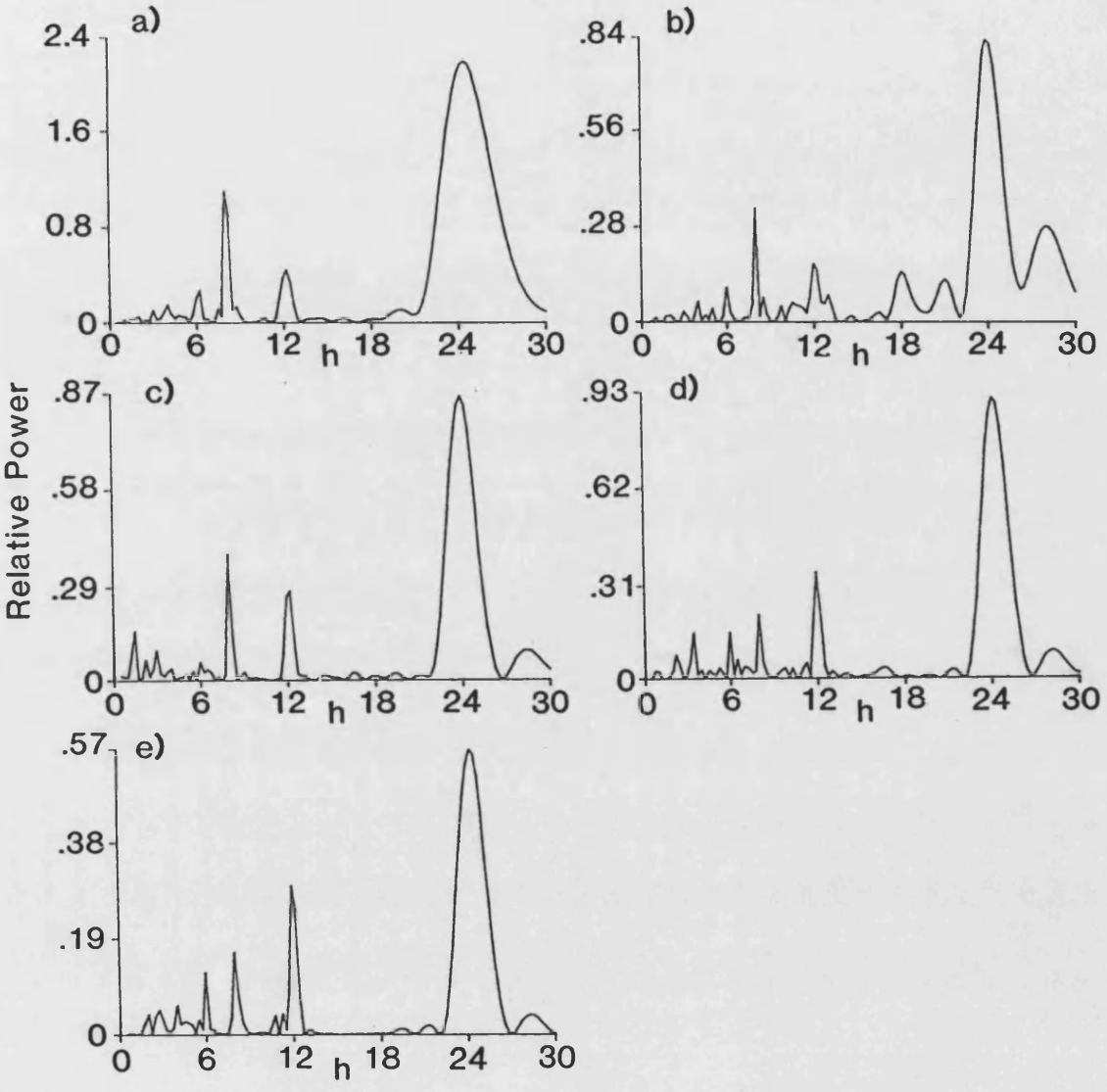
b) Constant darkness, days 13-22.

c) Constant darkness, days 23-32.

d) Constant darkness, days 33-42.

e) Constant darkness, days 43-52.

For a summary of the derived periodogram function, see Table 9.1 (animal 2).


a)
2.4
1.6
0.8
0
b)
.84
.56
.28
0
c)
.87
.58
.29
0
d)
.93
.62
.31
0
e)
.57
.38
.19
0
0 6 12 h 18 24 30
Relative Power

Group	Lighting Conditions (days)	Predominant Periods (h)	
1	L:D (1-10)	(8.00)	24.00
	D:D (12-21)		24.50
	D:D (24-33)		24.50
	D:D (34-43)		24.75
	D:D (45-54)		25.00
2	L:D (1-10)	(8.00,12.00, 21.25)	24.00
	D:D (12-21)		24.50
	D:D (24-33)		24.75
	D:D (34-43)		24.75
	D:D (45-54)		24.75

Table 9.2 Periodogram analysis of the circadian rhythm of locomotor activity expressed by untreated grouped rats.

L:D, normal lighting (12h light/12h dark) days 1-11; D:D, constant low-intensity red light from day 12; values in parenthesis indicate days used for spectral analysis.

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for group 1 are shown in Figs 9.3 and 9.4 respectively.

Figure 9.3 Actogram of the circadian rhythm of locomotor activity expressed by untreated grouped (N=3) rats.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Normal lighting (12h light/12h dark, lights on 0800), days 1-11.
Constant low-intensity red light, days 12-58.
Normal lighting (as above), days 59-62.

ACTOGRAM

BOX 8

THRESHOLD=50% SAMPLING INTERVAL=15Min

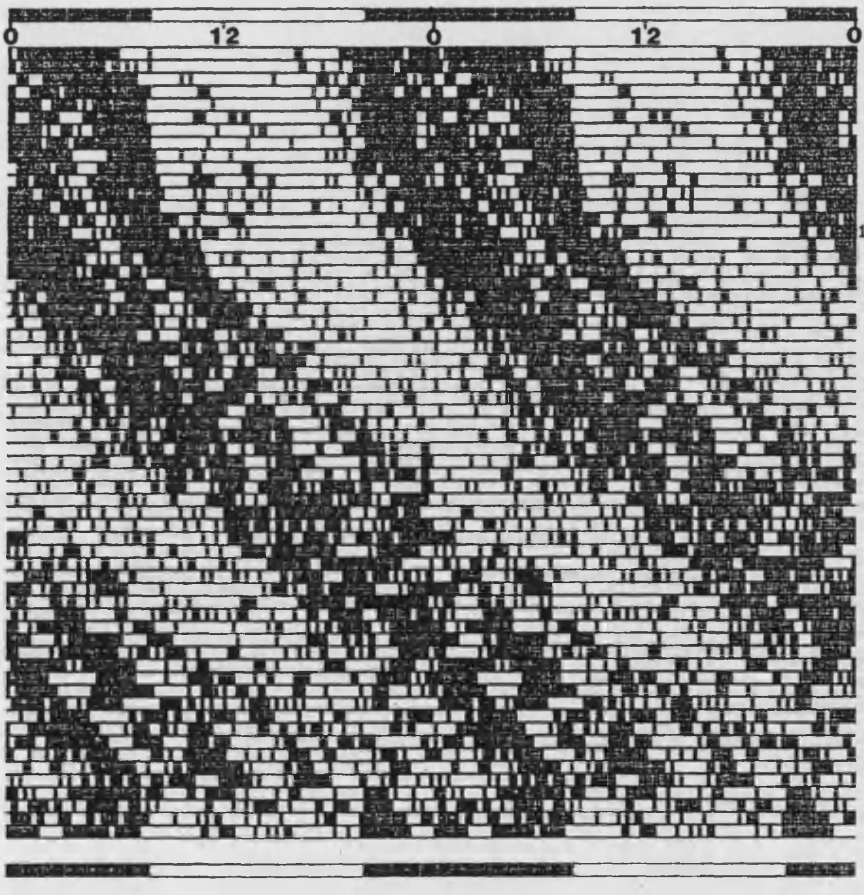


Figure 9.4 Periodogram analysis : Circadian locomotor activity of untreated grouped rats (N=3).

For actogram, see Fig. 9.3.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{4}$).

a) Normal daylight (12h light/12h dark), days 1-10.

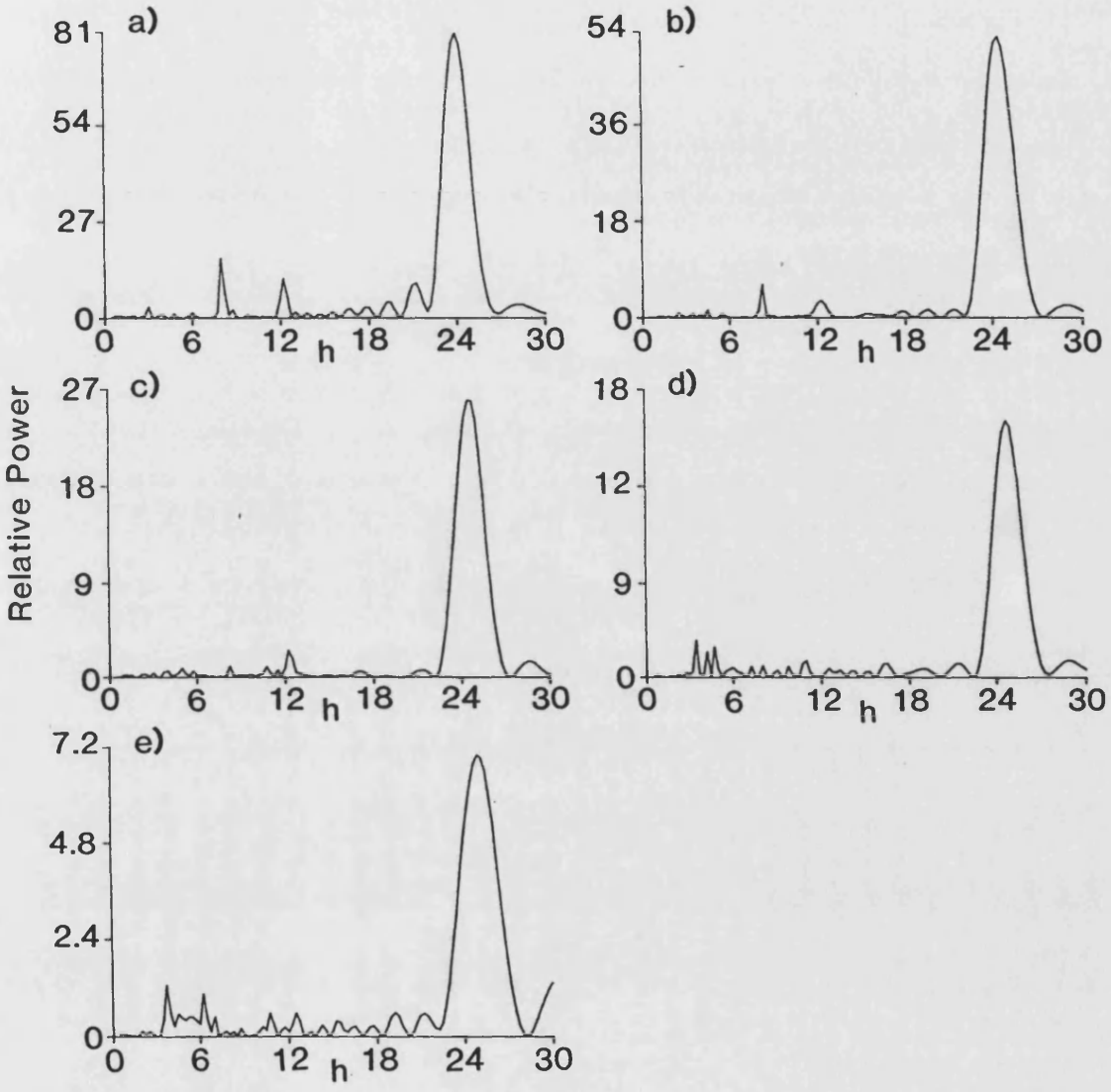
b) Constant low-intensity red light, days 12-21.

c) Constant low-intensity red light, days 24-33.

d) Constant low-intensity red light, days 34-43.

e) Constant low-intensity red light, days 45-54.

For a summary of the derived periodogram function, see Table 9.2 (group 1).


Relative Power
a)
81
54
27
0
b)
54
36
18
0
c)
27
18
9
0
d)
18
12
9
0
e)
7.2
4.8
2.4
0
0 6 12 h 18 24 30

9.3.2 Clomipramine

9.3.2.1 Individual animals

Visual inspection of the actograms and periodogram analysis (an example of which is given in Fig. 9.5 together with the derived periodograms in Fig. 9.6) suggests that the fundamental period of circadian locomotor activity was essentially maintained during chronic clomipramine treatment (Table 9.3). The actograms provide no evidence of phase-shift of the circadian locomotor activity induced by the onset of clomipramine treatment. Compared to the pre-treatment period, however, periodogram analysis suggests that the circadian rhythm of locomotor activity became slightly disrupted during clomipramine treatment as indicated by the occurrence of activity peaks with a period shorter than and including 16h. (Table 9.3). Following clomipramine treatment the disruption of the circadian rhythm became even more marked.

9.3.2.2 Grouped animals

Inspection of the actograms and periodogram analysis suggests that chronic clomipramine treatment of grouped animals induced a gradual, progressive, disruption of the circadian rhythm of locomotor activity, which was especially marked during the second 10-day period of drug treatment, without modifying the fundamental period of the circadian rhythm (Table 9.4; see also the example actogram and the derived periodograms provided in Figs. 9.7 and 9.8 respectively). Closer inspection of the actograms suggests that the disruption of locomotor activity occurred from about day 7 of clomipramine treatment (Fig. 9.7). Following clomipramine treatment the disruption of the circadian rhythm was even more apparent. The actograms provided no evidence of phase-shift of the circadian

locomotor activity induced by the onset of clomipramine treatment.

Animal	Lighting Conditions (days)	Drug Treatment	Predominant Periods (h)	
1	D:D (4-13)	Pre	(4.00, 4.75, 8.00)	24.00
	D:D (15-24)	1-10	(2.25, 4.75, 8.00) (12.00)	23.75
	D:D (25-34)	11-20	(4.00, 11.75, 16.00)	24.00
	D:D (36-45)	Post	(4.00, 4.75, 6.00) (18.75)	24.50
2	D:D (4-13)	Pre	(4.00)	23.75
	D:D (15-24)	1-10	(4.75, 8.00, 12.00)	23.75
	D:D (25-34)	11-20	(4.00, 8.00, 11.75)	24.00
	D:D (36-45)	Post	(4.00, 6.00, 9.50) (19.25)	24.00

Table 9.3 Periodogram analysis of the effect of chronic clomipramine, 20 mg Kg^{-1} day^{-1} po, on the circadian rhythm of locomotor activity expressed by individual rats.

D:D, constant darkness from day 4; values in parenthesis indicate days used for spectral analysis.

Drug treatment days 14-35 inclusive.

Pre, pre-treatment period.

1-10, 11-20; period of drug treatment.

Post, post-treatment period.

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for animal 2 are shown in Figs 9.5 and 9.6 respectively.

Figure 9.5 Actogram of the circadian rhythm of locomotor activity expressed by an isolated rat treated chronically with clomipramine, 20 mg Kg^{-1} day^{-1} po.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Normal lighting (12h light/12h dark, lights on 0800), days 1-3.
Constant darkness, days 4-55.
Normal lighting (as above), days 56-60.
Drug treatment, days 14-35 inclusive.

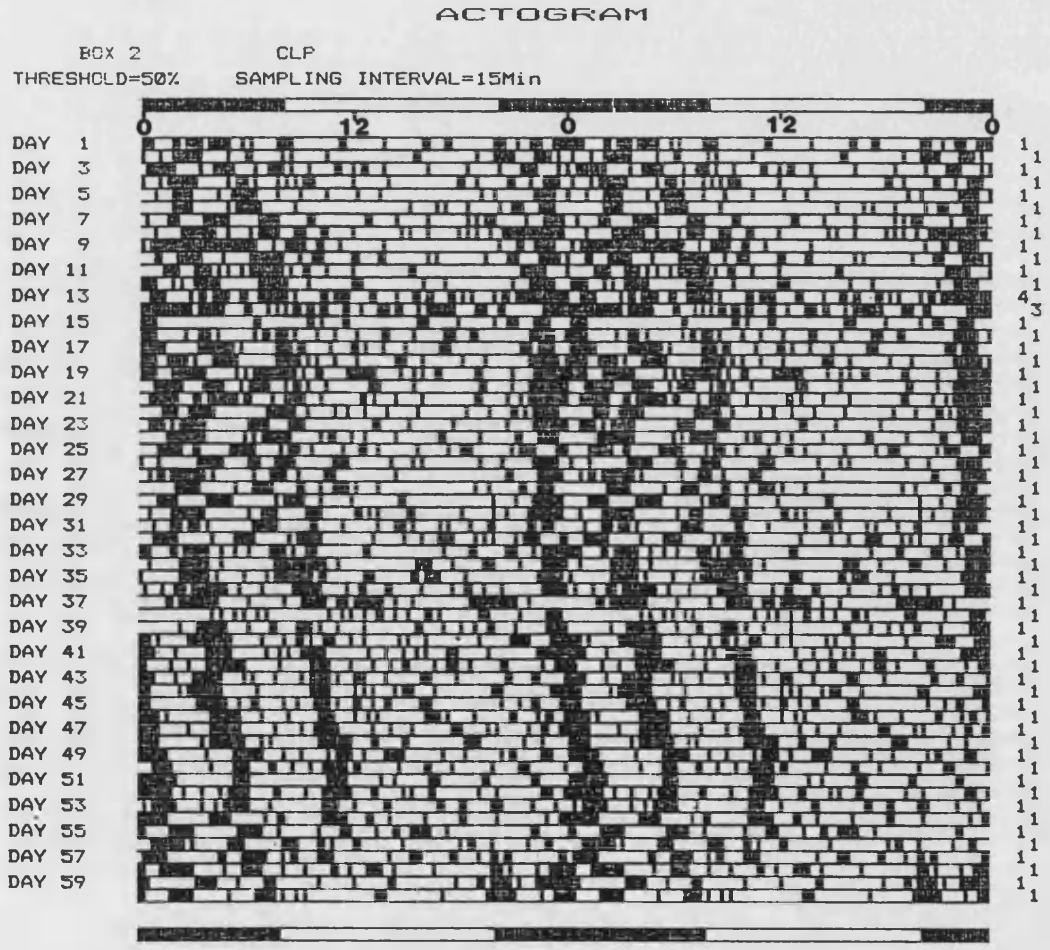

ACTOGRAM
BOX 2 CLP
THRESHOLD=50% SAMPLING INTERVAL=15Min
0 12 0 12 0
DAY 1
DAY 3
DAY 5
DAY 7
DAY 9
DAY 11
DAY 13
DAY 15
DAY 17
DAY 19
DAY 21
DAY 23
DAY 25
DAY 27
DAY 29
DAY 31
DAY 33
DAY 35
DAY 37
DAY 39
DAY 41
DAY 43
DAY 45
DAY 47
DAY 49
DAY 51
DAY 53
DAY 55
DAY 57
DAY 59

Figure 9.6 Periodogram analysis : Circadian locomotor activity of isolated rats treated chronically with clomipramine, 20 mg Kg^{-1} day^{-1} po.

For actogram, see Fig. 9.5.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{4}$).

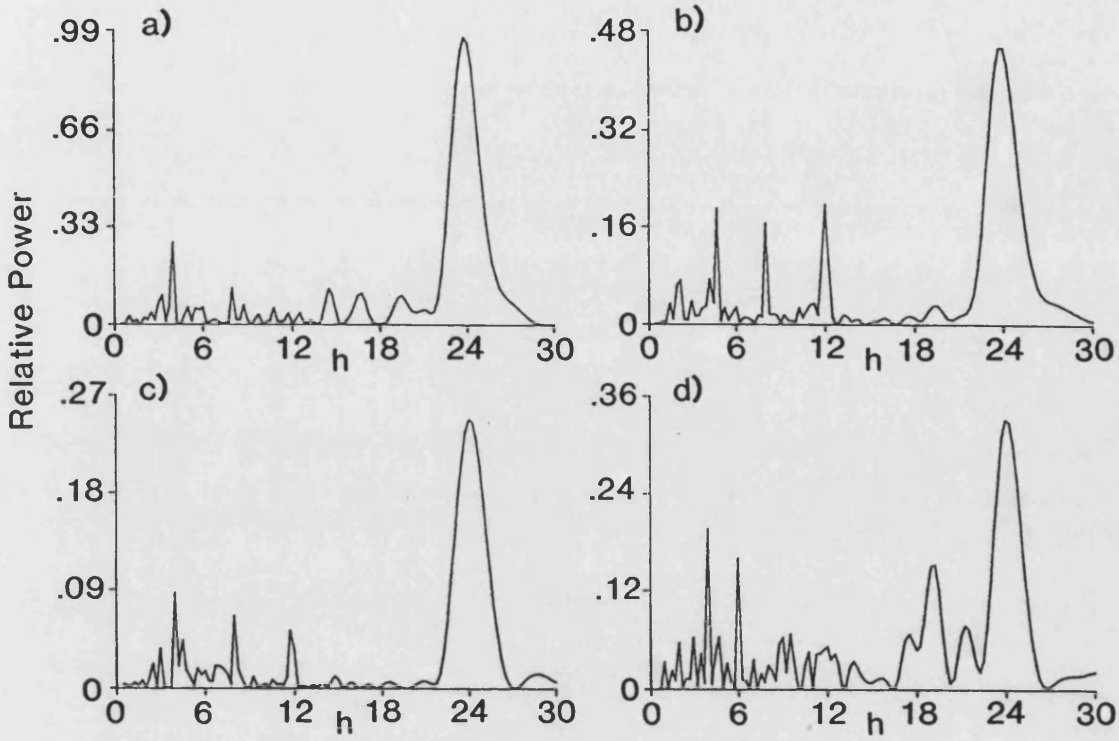
a) Constant darkness, days 4-13 (pre-treatment).

b) Constant darkness, days 15-24 (drug treatment days 1-10).

c) Constant darkness, days 25-34 (drug treatment days 11-20).

d) Constant darkness, days 36-45 (post-treatment).

For a summary of the derived periodogram function, see Table 9.3 (animal 2).


Relative Power
a)
.99
.66
.33
0
b)
.48
.32
.16
0
c)
.27
.18
.09
0
d)
.36
.24
.12
0
0 6 12 h 18 24 30

Group	Lighting Conditions (days)	Drug Treatment	Predominant Periods (h)	
1	L:D (1-10)		(8.00)	24.00
	D:D (12-21)	Pre	(8.25)	24.25
	D:D (24-33)	1-10	(8.25)	24.75
	D:D (34-43)	11-20	(4.00, 8.25)	25.00
	D:D (45-54)	Post	(3.50, 4.00, 4.75) (6.00, 12.00)	24.75
2	L:D (1-10)			24.00
	D:D (12-21)	Pre		24.75
	D:D (24-33)	1-10		24.50
	D:D (34-43)	11-20	(3.50, 4.25, 4.75) (12.25)	24.25
	D:D (45-54)	Post	(3.50, 4.25)	24.25

Table 9.4 Periodogram analysis of the effect of chronic clomipramine, 20 mg Kg^{-1} day^{-1} po, on the circadian rhythm of locomotor activity expressed by grouped rats.

L:D, normal lighting (12h light/12h dark) days 1-10; D:D, constant low-intensity red light from day 11; values in parenthesis indicate experimental days used for spectral analysis.

Drug treatment days 23-44 inclusive.

Pre, pre-treatment period.

1-10, 11-20, period of drug treatment.

Post, post-treatment period.

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for group 1 are shown in Figs 9.7 and 9.8 respectively.

Figure 9.7 Actogram of the circadian rhythm of locomotor activity expressed by grouped rats (N=3) treated chronically with clomipramine, 20 mg Kg^{-1} day^{-1} po.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Normal lighting (12h light/12h dark, lights on 0800), days 1-10.
Constant low-intensity red light, days 11-58.
Normal lighting (as above), days 59-62.
Drug treatment, days 23-44 inclusive.

ACTOGRAM

BOX 8

THRESHOLD=50% SAMPLING INTERVAL=15Min

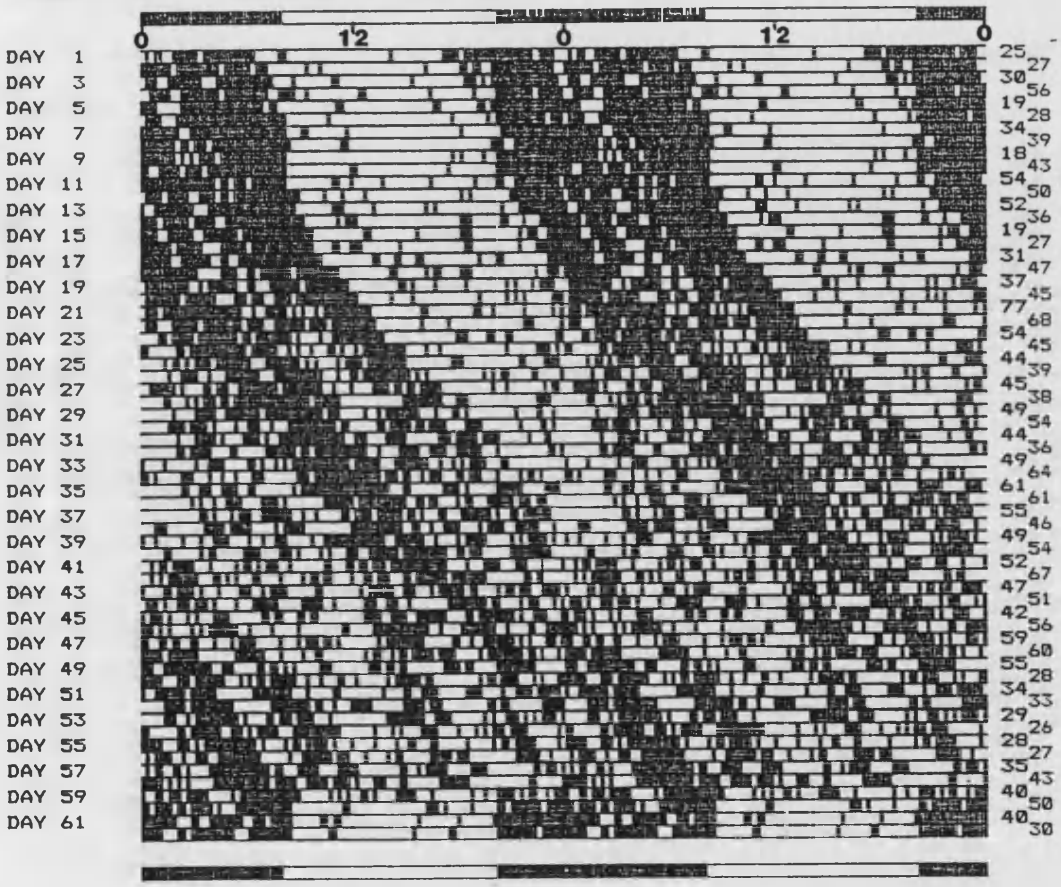


Figure 9.8 Periodogram analysis : Circadian locomotor activity of grouped rats (N=3) treated chronically with clomipramine, 20 mg Kg^{-1} day^{-1} po.

For actogram, see Fig. 9.7.

Abscissa: Period length (h). Ordinate: Relative power (*10^{4}).

a) Normal daylight (12h light/12h dark), days 1-10.

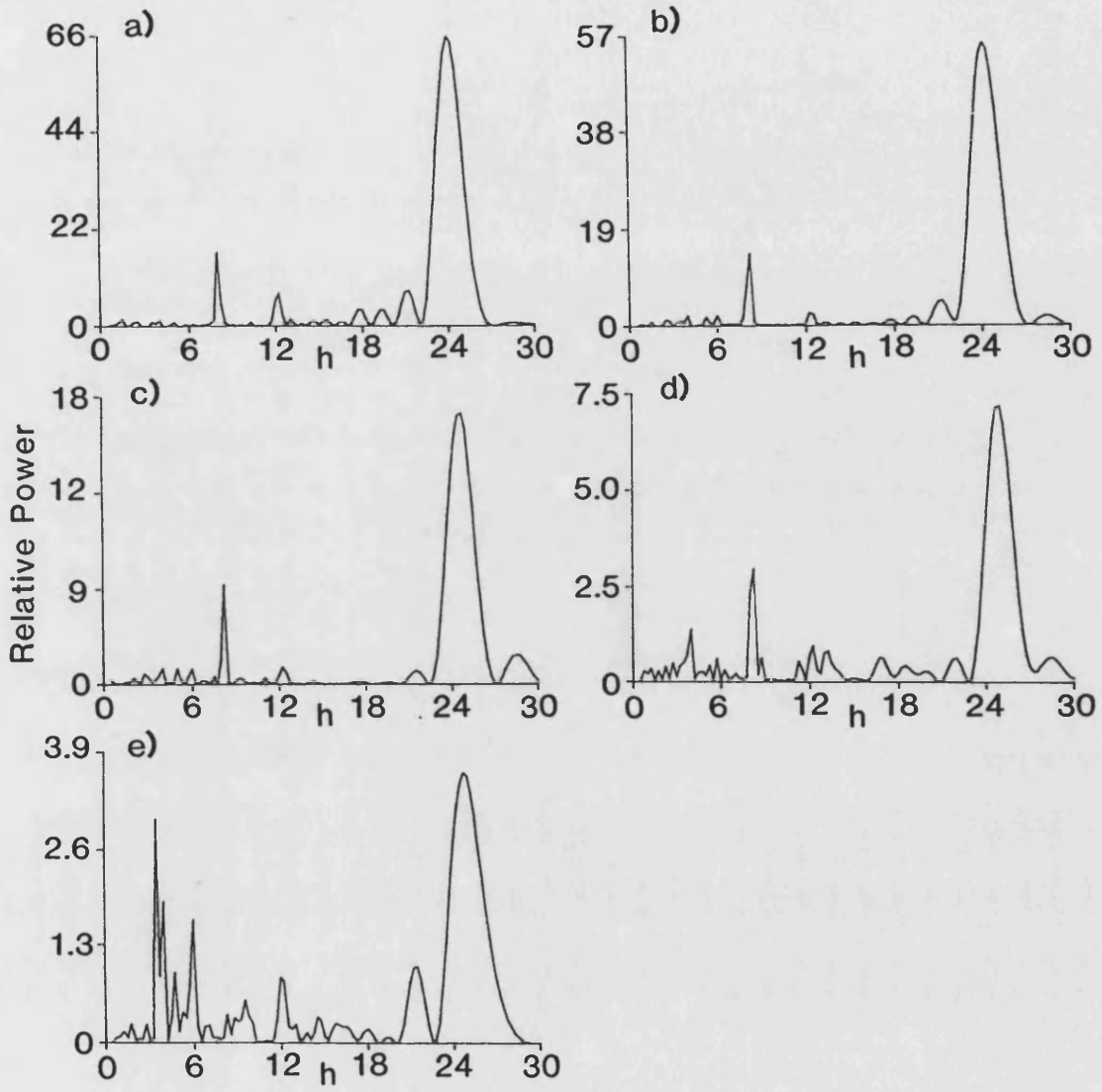
b) Constant low-intensity red light, days 12-21 (pre-treatment).

c) Constant low-intensity red light, days 24-33 (drug treatment days 1-10).

d) Constant low-intensity red light, days 34-43 (drug treatment days 11-20).

e) Constant low-intensity red light, days 45-54 (post-treatment).

For a summary of the derived periodogram function, see Table 9.4 (group 1).


a)
66
44
22
0
b)
57
38
19
0
c)
18
12
9
0
d)
7.5
5.0
2.5
0
e)
3.9
2.6
1.3
0
0 6 12 h 18 24 30
Relative Power

9.3.3 Fluoxetine

9.3.3.1 Individual animals

Inspection of the actograms and periodogram analysis suggests that chronic fluoxetine treatment of isolated animals induced a slight disruption of the circadian rhythm of locomotor activity during the first 20 days of treatment with little or no effect on the fundamental period length (see example actogram and derived periodograms provided in Figs. 9.9 and 9.10 respectively and Table 9.5). From 21 to 30 days of drug treatment, however, the disruption of the circadian rhythm of locomotor activity was more marked as indicated by the numerous activity peaks of period length less than and including 21.25h., together with a slight increase in the fundamental period length (Table 9.5). During the 10-day period following the cessation of drug treatment periodogram analysis suggests that the circadian rhythm of locomotor activity splits into two rhythms of 6h and 24h. period length. The actograms provided no evidence of phase-shift of the circadian locomotor activity induced by the onset of fluoxetine treatment.

9.3.3.2 Grouped animals

Chronic fluoxetine treatment of grouped animals had little or no effect upon the circadian rhythm of locomotor activity during the first 20 days of treatment (see example actogram and derived periodograms provided in Figs. 9.11 and 9.12 respectively and Table 9.6). From 21 to 30 days of drug treatment, however, the circadian rhythm of locomotor activity was markedly disrupted; which in one group of animals resulted in primary rhythms of 8.25h., 12.5h. and 24.25h. (see actogram and derived periodograms provided in Figs. 9.13 and 9.14 respectively and Table 9.6). Periodogram analysis of the

initial 10-day period following drug treatment suggests that the circadian rhythm regained some degree of stability compared to the level of disruption observed during the latter stages of chronic drug treatment (see Figs. 9.12 and 9.14). The actograms provided no evidence of phase-shift of the circadian locomotor activity induced by the onset of fluoxetine treatment.

Animal	Lighting Conditions (days)	Drug Treatment	Predominant Periods (h)	
1	D:D (4-13)	Pre	(8.00)	23,75
	D:D (15-24)	1-10		23.75
	D:D (25-34)	11-20		23.75
	D:D (35-44)	21-30		24.00
	D:D (46-55)	Post		24.00
2	D:D (4-13)	Pre	(8.00)	23.75
	D:D (15-24)	1-10	(8.00)	24.00
	D:D (25-34)	11-20		24.00
	D:D (35-44)	21-30	(4.00, 6.00, 7.25) (8.25, 10.00, 12.25) (15.50, 19.00, 21.25)	24.50
	D:D (46-55)	Post	(4.00, 12.00)	6.00 24.00

Table 9.5 Periodogram analysis of the effect of chronic fluoxetine, 2 mg Kg^{-1} day^{-1} po, on the circadian rhythm of locomotor activity expressed by individual rats.

D:D, constant darkness from day 4; values in parenthesis indicate days used for spectral analysis.

Drug treatment days 14-45 inclusive.

Pre, pre-treatment period.

1-10, 11-20, 21-30; period of drug treatment.

Post, post-treatment period.

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for animal 2 are shown in Figs 9.9 and 9.10 respectively.

Figure 9.9 Actogram of the circadian rhythm of locomotor activity expressed by an isolated rat treated chronically with fluoxetine, 2 mg Kg^{-1} day^{-1} po.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Normal lighting (12h light/12h dark, lights on 0800), days 1-3.
Constant darkness, days 4-55.
Normal lighting (as above), days 56-60.
Drug treatment, days 14-45 inclusive.

ACTOGRAM

BOX 6 FLU

THRESHOLD=50% SAMPLING INTERVAL=15Min

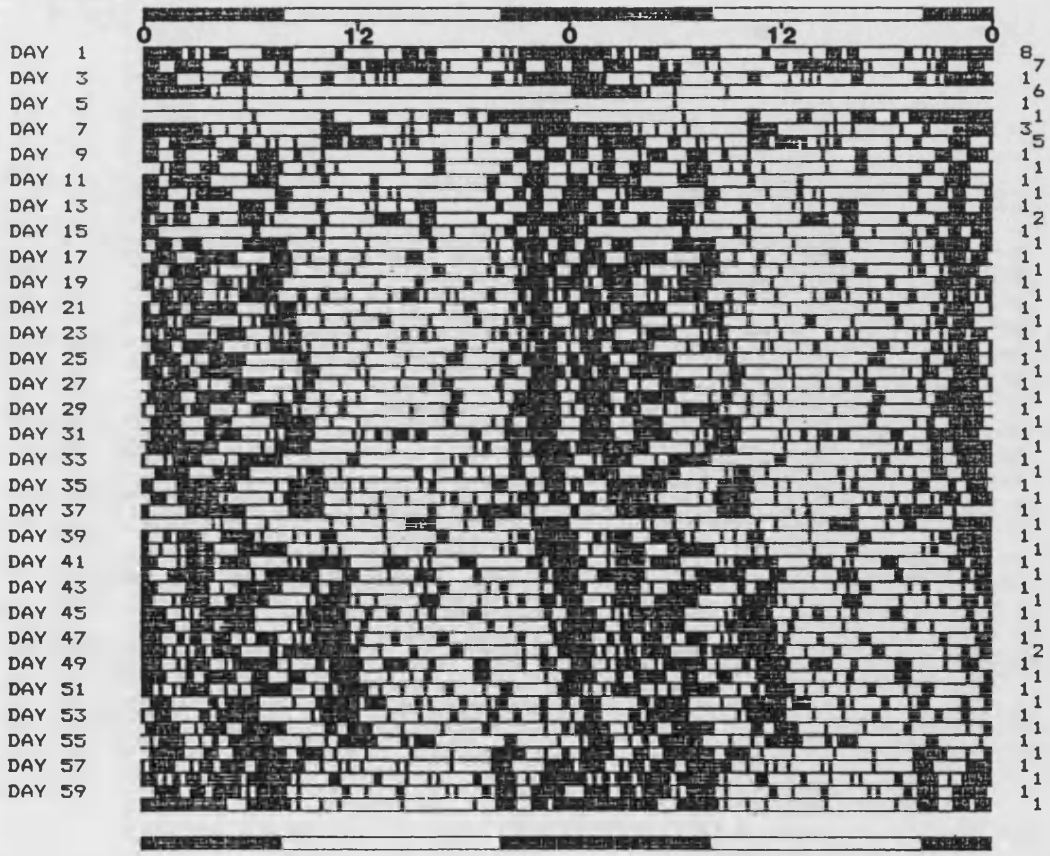


Figure 9.10 Periodogram analysis : Circadian locomotor activity of an isolated rat treated chronically with fluoxetine, 2 mg Kg^{-1} day^{-1} po.

For actogram, see Fig. 9.9.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{4}$).

a) Constant darkness, days 4-13 (pre-treatment).

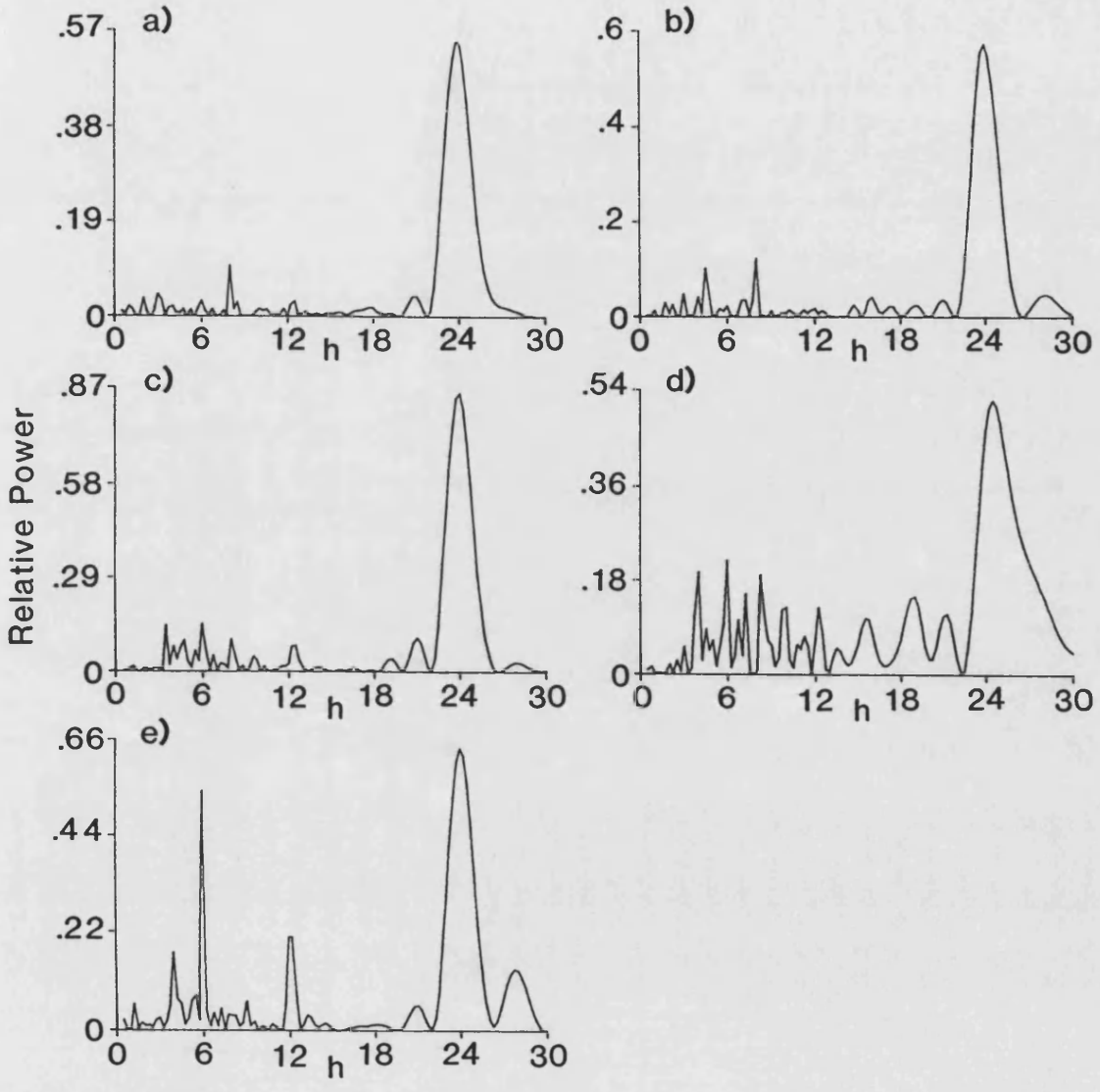
b) Constant darkness, days 15-24 (drug treatment days 1-10).

c) Constant darkness, days 25-34 (drug treatment days 11-20).

d) Constant darkness, days 35-44 (drug treatment days 21-30).

e) Constant darkness, days 46-55 (post-treatment).

For a summary of the derived periodogram function, see Table 9.5 (animal 2).


Relative Power
a)
.57
.38
.19
0
b)
.6
.4
.2
0
c)
.87
.58
.29
0
d)
.54
.36
.18
0
e)
.66
.44
.22
0
0 6 12 h 18 24 30

Group	Lighting Conditions (days)	Drug Treatment	Predominant Periods (h)	
1	D:D (5-14)	Pre		24.50
	D:D (16-25)	1-10		25.00
	D:D (26-35)	11-20		24.75
	D:D (36-45)	21-30	(4.25)	24.50
	D:D (48-57)	Post		24.75
2	D:D (1-10)	Pre		25.00
	D:D (12-21)	1-10		24.75
	D:D (22-31)	11-20	(5.00, 28.75)	24.75
	D:D (32-41)	21-30	(6.25)	8.25
				12.50
				24.25
	D:D (42-51)	Post	(8.25, 25.50)	12.25
3*	D:D (5-14)	Pre	(8.25, 12.25)	24.50
	D:D (16-25)	1-10		24.75
	D:D (26-35)	11-20		24.75
	D:D (36-45)	21-30		24.50
	D:D (48-57)	Post		24.75

Table 9.6 Periodogram analysis of the effect of chronic fluoxetine, 2 or 6(*) mg Kg^{-1} day^{-1} po, on the circadian rhythm of locomotor activity expressed by grouped rats.

D:D, constant low-intensity red light from day 1; values in parenthesis indicate days used for spectral analysis.

Drug treatment days 15-47 inclusive groups 1 and 3, days 12-41 inclusive group 2.

Pre, pre-treatment period.

1-10, 11-20, 21-30; period of drug treatment.

Post, post-treatment period.

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for groups 1 and 2 are shown in Figs 9.11 and 9.12, and Figs 9.13 and 9.14 respectively.

Figure 9.11 Actogram of the circadian rhythm of locomotor activity expressed by grouped rats (N=3) treated chronically with fluoxetine, 2 mg Kg^{-1} day^{-1} po.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Constant low-intensity red light, days 1-59.
Reverse lighting (12h light/12h dark, lights on 2000), days 60-63.
Drug treatment, days 15-47 inclusive.

ACTOGRAM

BOX 8

THRESHOLD=50% SAMPLING INTERVAL=15Min

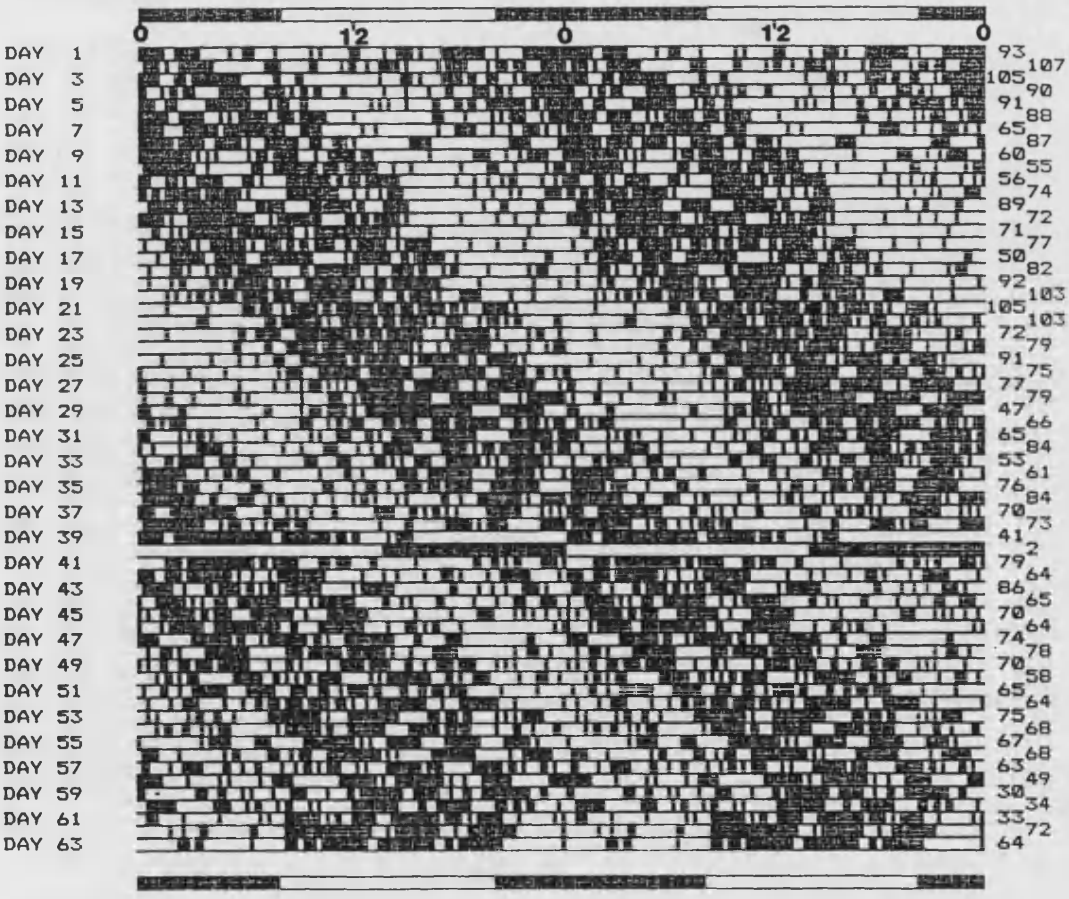


Figure 9.12 Periodogram analysis : Circadian locomotor activity of grouped rats (N=3) treated chronically with fluoxetine, 2 mg Kg^{-1} day^{-1} po.

For actogram, see Fig. 9.11.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{4}$).

a) Constant low-intensity red light, days 5-14 (pre-treatment).

b) Constant low-intensity red light, days 16-25 (drug treatment days 1-10).

c) Constant low-intensity red light, days 26-35 (drug treatment days 11-20).

d) Constant low-intensity red light, days 36-45 (drug treatment days 21-30).

e) Constant low-intensity red light, days 48-57 (post-treatment).

For a summary of the derived periodogram function, see Table 9.6 (group 1).

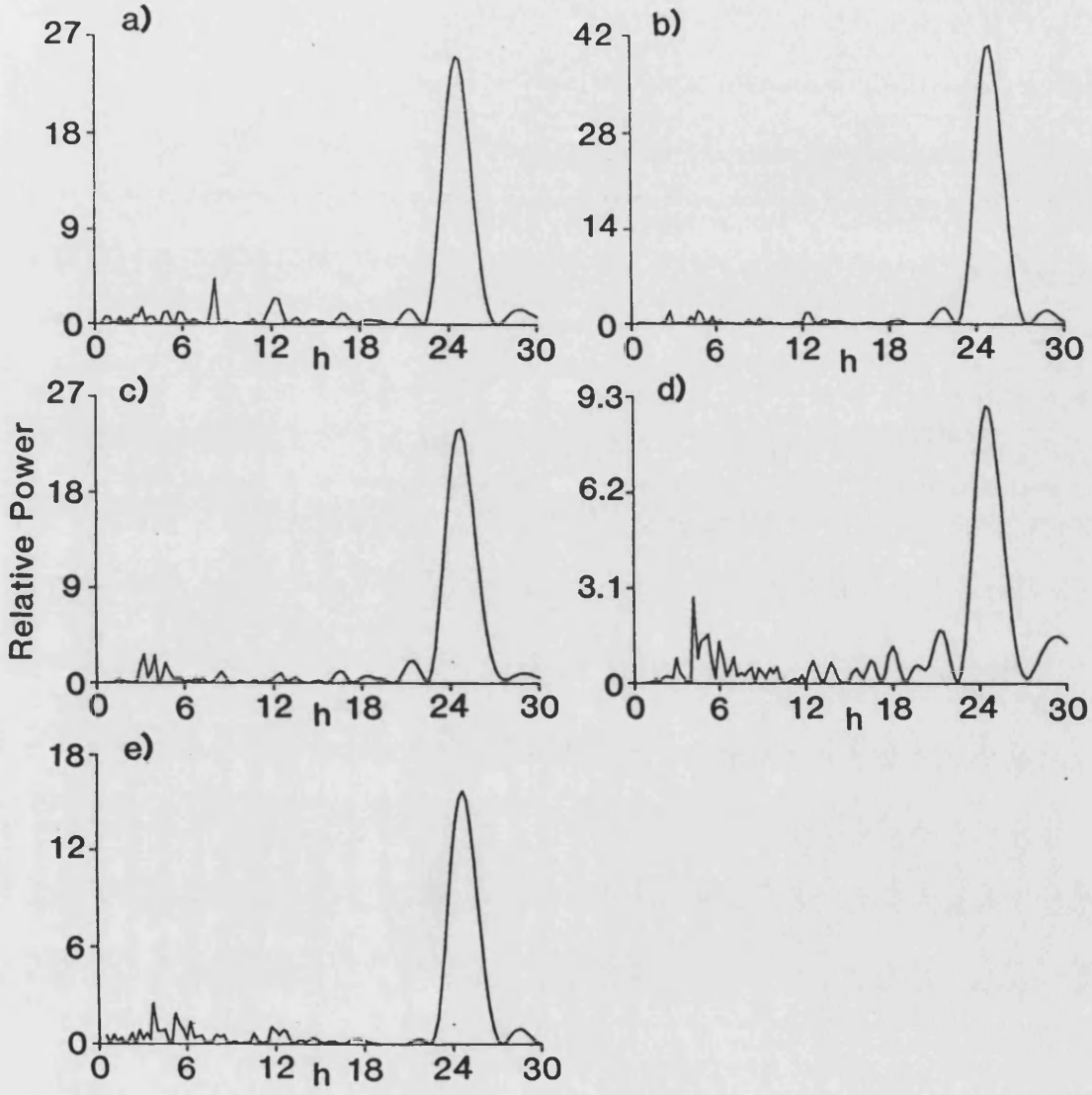

a)
b)
c)
d)
e)
Relative Power
h
0
6
12
18
24
30
27
18
9
42
28
14
9.3
6.2
3.1
12
6

Figure 9.13 Actogram of the circadian rhythm of locomotor activity expressed by grouped rats (N=3) treated chronically with fluoxetine, 2 mg Kg^{-1} day^{-1} po.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Constant low-intensity red light, days 1-51.
Drug treatment, days 12-41 inclusive.

ACTOGRAM

BOX 8

THRESHOLD=50% SAMPLING INTERVAL=15Min

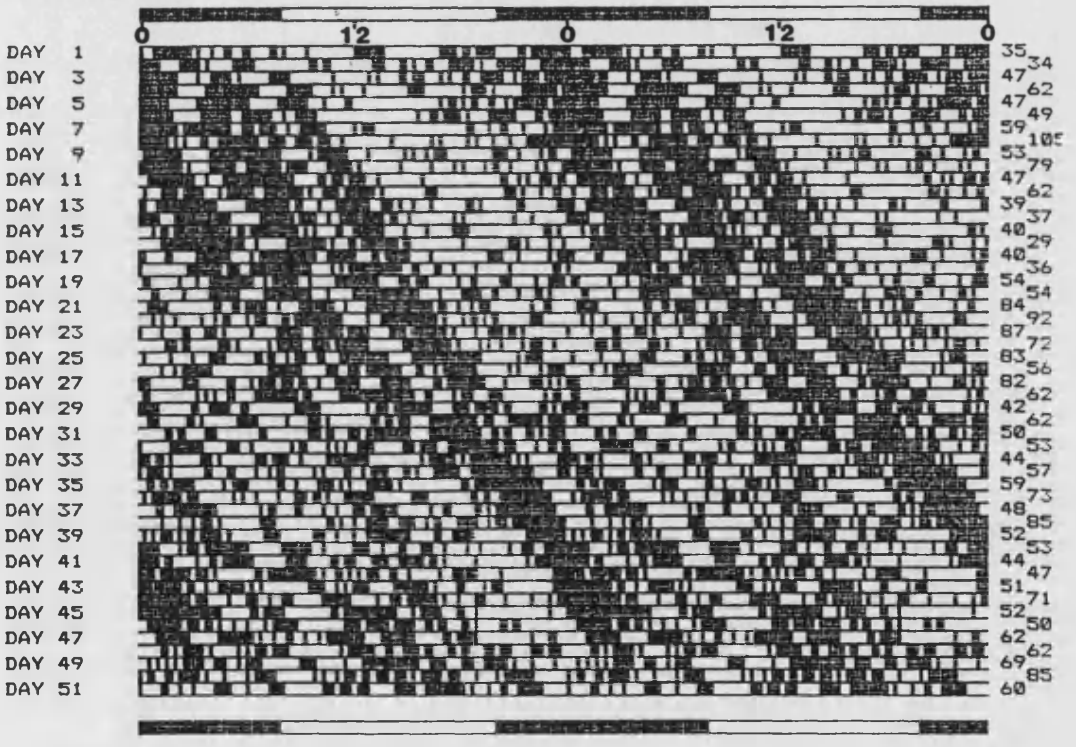


Figure 9.14 Periodogram analysis : Circadian locomotor activity of grouped rats (N=3) treated chronically with fluoxetine, 2 mg Kg^{-1} day^{-1} po.

For actogram, see Fig. 9.13.

Abscissa: Period length (h). Ordinate: Relative power (*10^{4}).

a) Constant low-intensity red light, days 1-10 (pre-treatment).

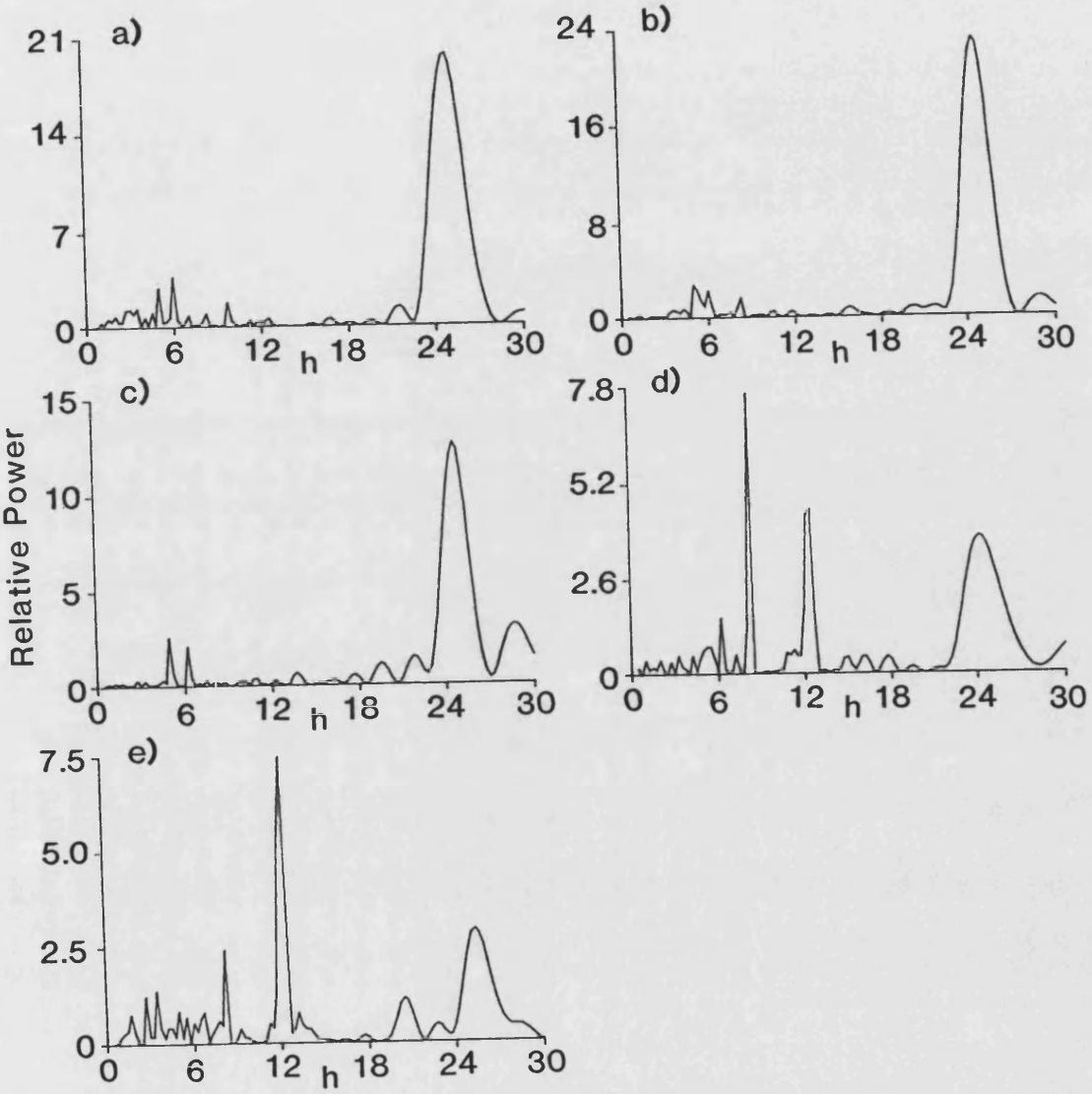
b) Constant low-intensity red light, days 12-21 (drug treatment days 1-10).

c) Constant low-intensity red light, days 22-31 (drug treatment days 11-20).

d) Constant low-intensity red light, days 32-41 (drug treatment days 21-30).

e) Constant low-intensity red light, days 42-51 (post-treatment).

For a summary of the derived periodogram function, see Table 9.6 (group 2).


a)
b)
c)
d)
e)
Relative Power
h
0
6
12
18
24
30
21
14
7
24
16
8
15
10
5
7.8
5.2
2.6
7.5
5.0
2.5

9.3.4 Mianserin

9.3.4.1 Individual animals

Chronic mianserin treatment had no effect on the circadian rhythm of locomotor activity exhibited by individual animals (see example actogram and derived periodograms provided in Figs. 9.15 and 9.16 respectively and Table 9.7). Following drug treatment, however, periodogram analysis suggests some disruption of the circadian rhythm. The actograms provided no evidence of phase-shift of the circadian locomotor activity induced by the onset of mianserin treatment.

9.3.4.2 Grouped animals

Chronic mianserin treatment of grouped animals had no effect on the circadian rhythm of locomotor activity during the period of drug treatment (see example actogram and derived periodograms provided in Figs. 9.17 and 9.18 respectively and Table 9.8). In one group of animals, however, there was some evidence of circadian rhythm disruption following drug treatment (see Table 9.8). The actograms provided no evidence of phase-shift of the circadian locomotor activity induced by the onset of mianserin treatment.

Animal	Lighting Conditions (days)	Drug Treatment	Predominant Periods (h)	
1	D:D (4-13)	Pre		23.75
	D:D (15-24)	1-10	(8.00)	23.75
	D:D (25-34)	11-20		24.00
	D:D (36-45)	Post	(4.75, 8.50, 9.00)	4.25
			(9.50, 13.75, 19.50)	24.75
2	D:D (4-13)	Pre	(3.00, 3.75, 4.75)	24.25
			(8.00, 11.75)	
	D:D (15-24)	1-10	(8.00, 12.00)	24.25
	D:D (25-34)	11-20	(6.00, 20.75)	23.75
	D:D (36-45)	Post	(4.00, 5.00,6.00)	24.50
			(8.50, 9.50, 12.00)	

Table 9.7 Periodogram analysis of the effect of chronic mianserin, 2 mg Kg^{-1} day^{-1} po, on the circadian rhythm of locomotor activity expressed by individual rats.

D:D, constant darkness from day 4; values in parenthesis indicate days used for spectral analysis.

Drug treatment days 14-35 inclusive.

Pre, pre-treatment period

1-10, 11-20; period of drug treatment.

Post, post-treatment period.

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for animal 2 are shown in Figs 9.15 and 9.16 respectively.

Figure 9.15 Actogram of the circadian rhythm of locomotor activity expressed by an isolated rat treated chronically with mianserin, 2 mg Kg^{-1} day^{-1} po.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Normal lighting (12h light/12h dark, lights on 0800), days 1-3.
Constant darkness, days 4-55.
Normal lighting (as above), days 56-60.
Drug treatment, days 14-35 inclusive.

ACTOGRAM

BOX 4 MIA

THRESHOLD=50% SAMPLING INTERVAL=15Min

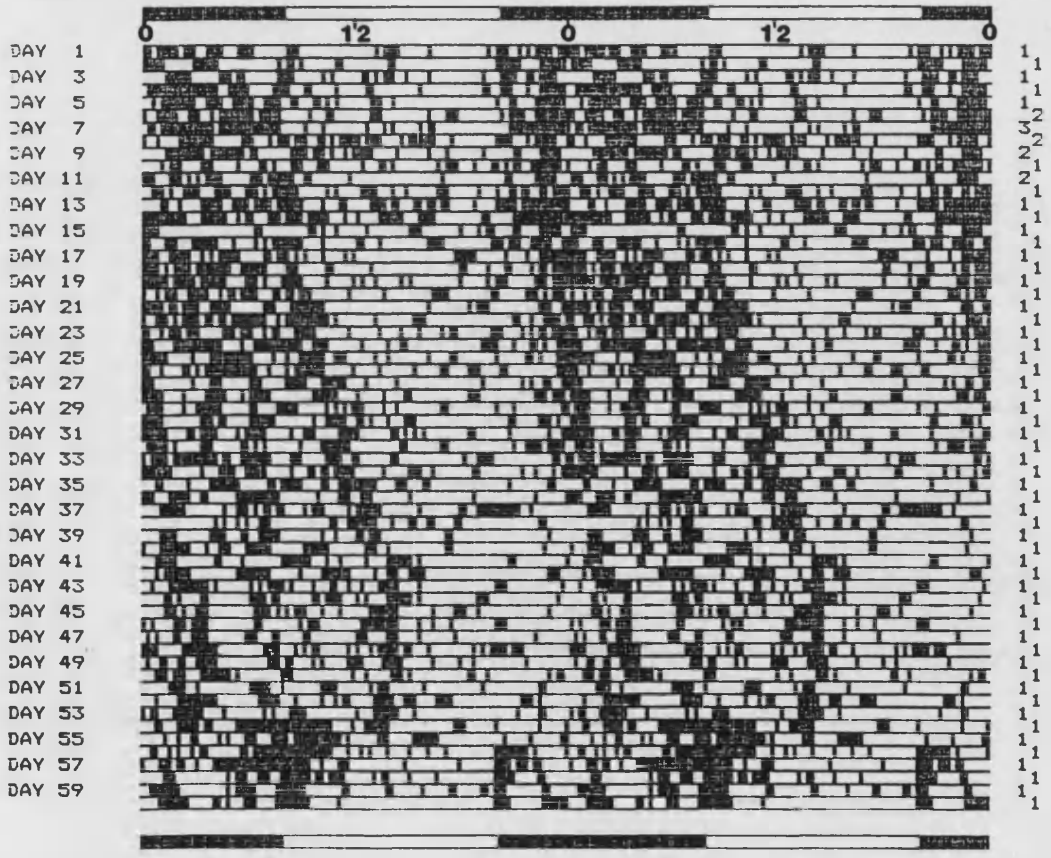


Figure 9.16 Periodogram analysis : Circadian locomotor activity of an isolated rat treated chronically with mianserin, 2 mg Kg^{-1} day^{-1} po.

For actogram, see Fig. 9.15.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{4}$).

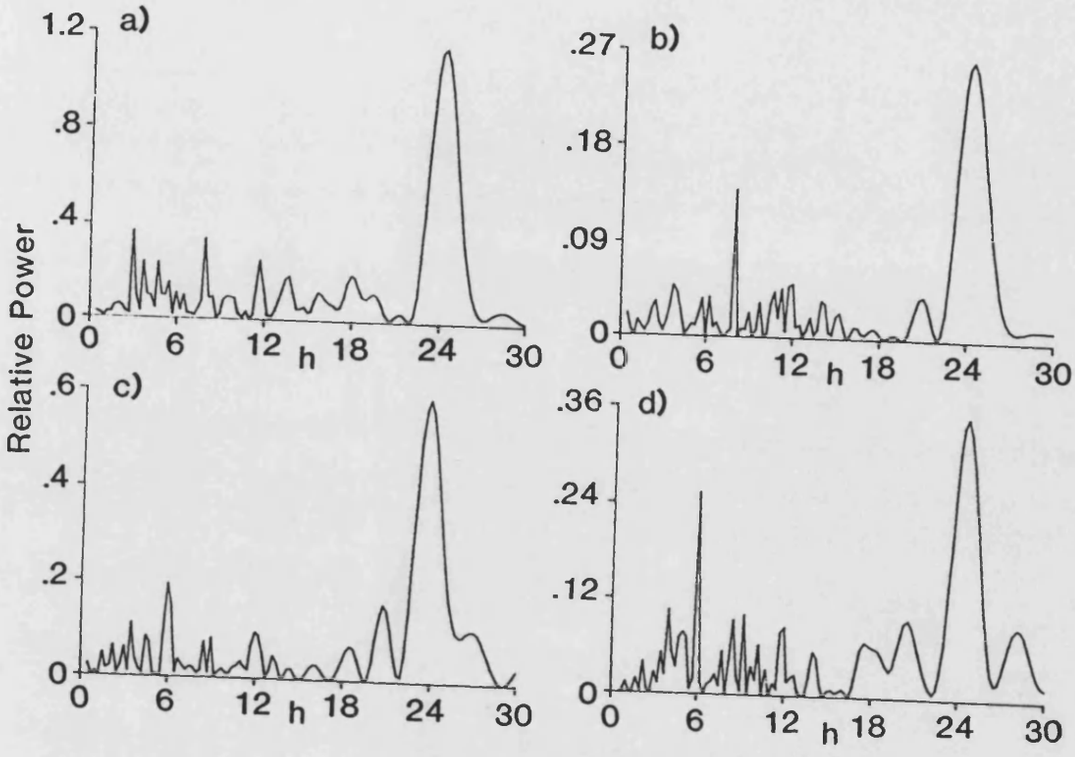
a) Constant darkness, days 4-13 (pre-treatment).

b) Constant darkness, days 15-24 (drug treatment days 1-10).

c) Constant darkness, days 25-34 (drug treatment days 11-20).

d) Constant darkness, days 36-45 (post-treatment).

For a summary of the derived periodogram function, see Table 9.7 (animal 2).


Relative Power
a)
1.2
.8
.4
0
b)
.27
.18
.09
0
c)
.6
.4
.2
0
d)
.36
.24
.12
0
0
6
12
h
18
24
30

Group	Lighting Conditions (days)	Drug Treatment	Predominant Periods (h)	
1	L:D (1-10)		(8.00, 12.25)	24.00
	D:D (12-21)	Pre	(8.25)	24.50
	D:D (24-33)	1-10		24.75
	D:D (34-43)	11-20		25.00
	D:D (45-54)	Post		25.00
2	L:D (1-10)		(8.00, 12.00)	24.00
	D:D (12-21)	Pre	(8.25, 12.25)	24.75
	D:D (24-33)	1-10		24.75
	D:D (34-43)	11-20	(8.25)	24.75
	D:D (45-54)	Post	(5.75, 6.25, 7.25) (12.25, 16.50, 18.00) (21.50)	23.75

Table 9.8 Periodogram analysis of the effect of chronic mianserin, 2 mg Kg^{-1} day^{-1} po, on the circadian rhythm of locomotor activity expressed by grouped rats.

L:D, normal lighting (12h light/12h dark) days 1-10; D:D, constant low-intensity red light from day 11; values in parenthesis indicate days used for spectral analysis.

Drug treatment days 23-44 inclusive.

Pre, pre-treatment period.

1-10, 11-20; period of drug treatment.

Post, post-treatment period.

Periodogram analysis; values indicate the fundamental and secondary (in parenthesis) periods of rhythmic activity.

The actogram and derived periodogram functions for group 1 are shown in Figs 9.17 and 9.18 respectively.

Figure 9.17 Actogram of the circadian rhythm of locomotor activity expressed by grouped rats (N=3) treated chronically with mianserin, 2 mg Kg^{-1} day^{-1} po.
Solid blocks indicate where the activity counts per epoch (15 min) were equal to or greater than the median threshold indicated to the right of the actogram.

Normal lighting (12h light/12h dark, lights on 0800), days 1-10.
Constant low-intensity red light, days 11-57.
Drug treatment, days 23-44 inclusive.

ACTOGRAM

BOX 8

THRESHOLD=50% SAMPLING INTERVAL=15Min

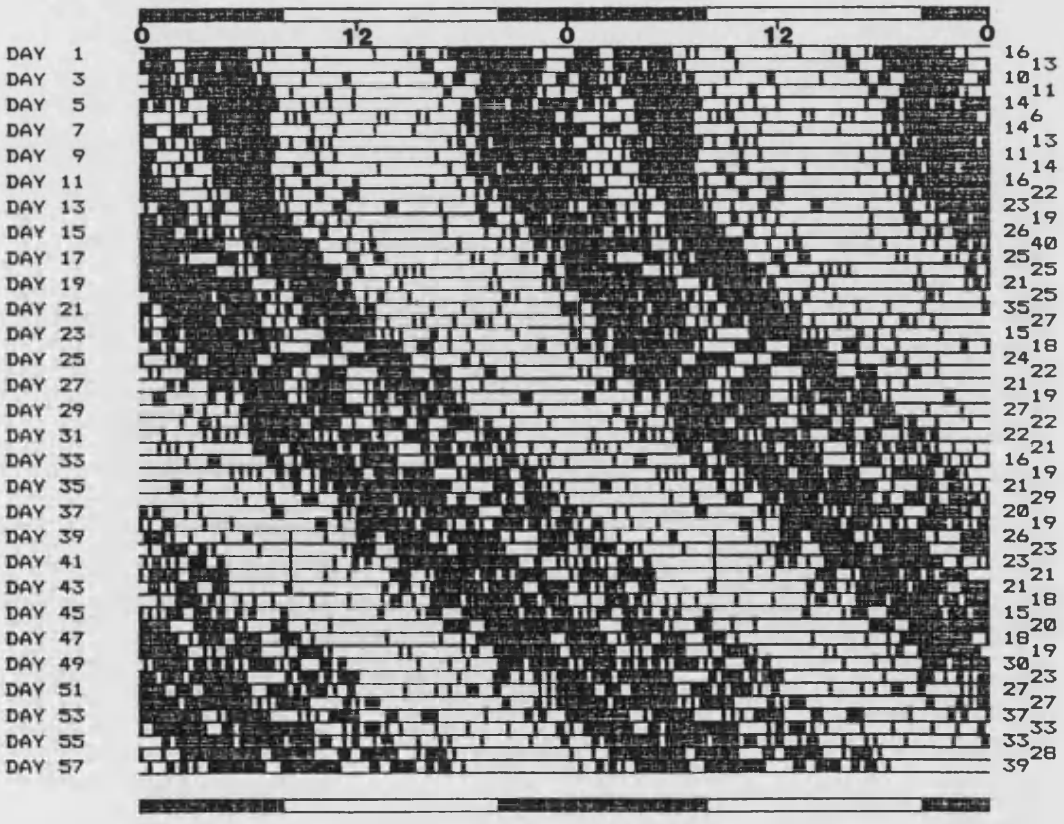


Figure 9.18 Periodogram analysis : Circadian locomotor activity of grouped rats (N=3) treated chronically with mianserin, 2 mg Kg^{-1} day^{-1} po.

For actogram, see Fig. 9.17.

Abscissa: Period length (h). Ordinate: Relative power ($*10^4$).

a) Normal daylight (12h light/12h dark), days 1-10.

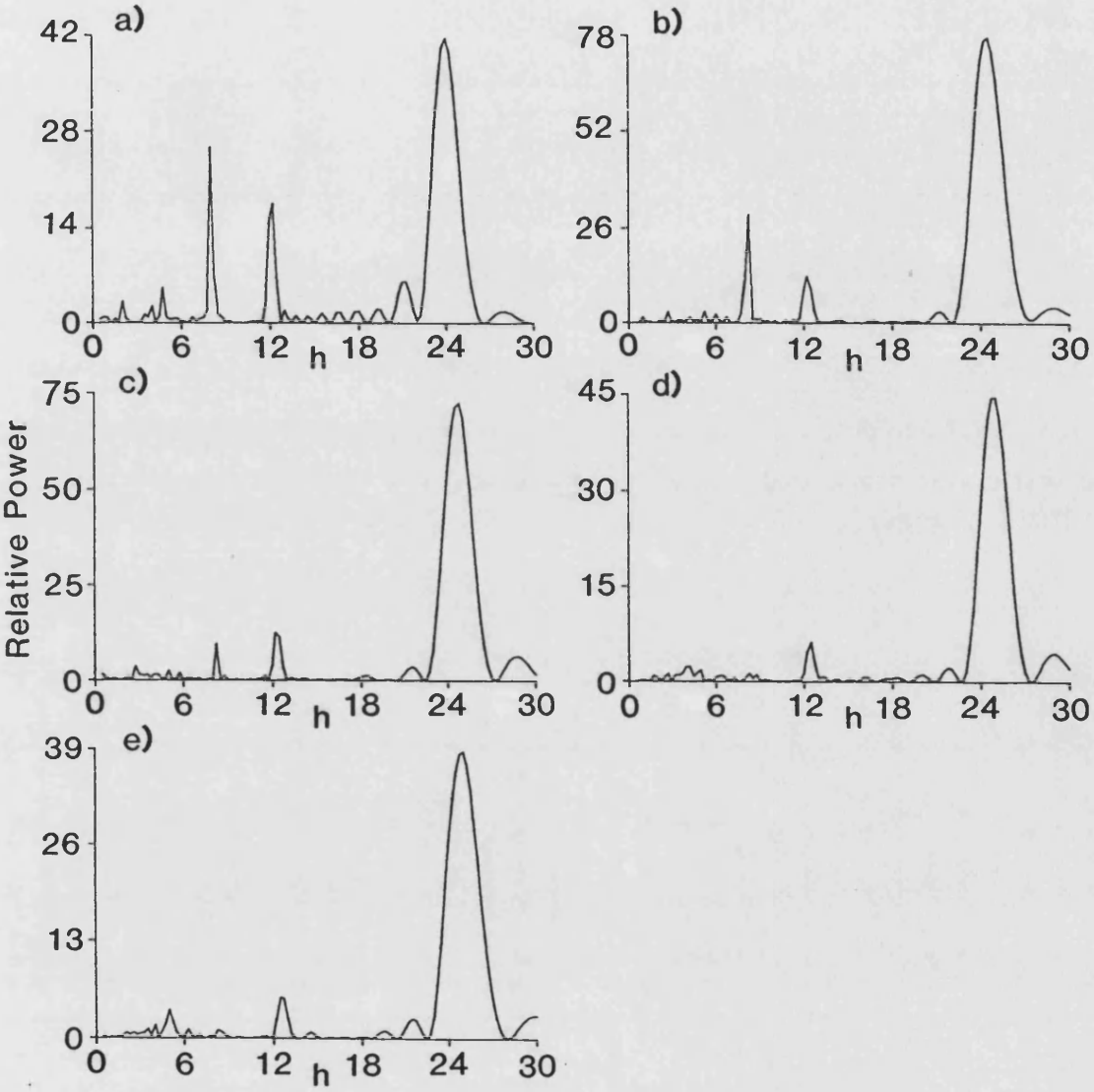
b) Constant low-intensity red light, days 12-21 (pre-treatment).

c) Constant low-intensity red light, days 24-33 (drug treatment days 1-10).

d) Constant low-intensity red light, days 34-43 (drug treatment days 11-20).

e) Constant low-intensity red light, days 45-54 (post-treatment).

For a summary of the derived periodogram function, see Table 9.8 (group 1).


a)
42
28
14
0
0 6 12 h 18 24 30
b)
78
52
26
0
0 6 12 h 18 24 30
c)
75
50
25
0
0 6 12 h 18 24 30
d)
45
30
15
0
0 6 12 h 18 24 30
e)
39
26
13
0
0 6 12 h 18 24 30
Relative Power

9.4 Discussion

The aim of these studies was to test the hypothesis that an ability to modify the free-running circadian locomotor activity rhythms of rats was shared by the antidepressants clomipramine, fluoxetine and mianserin. Apart from relying on visual inspection of the resulting actograms to identify changes in the circadian rhythmicity of locomotor activity, the data was also subjected to periodogram analysis to determine the periodicity of the rhythmic components. In general the fundamental period of rhythmic locomotor activity determined by periodogram analysis agreed with inspection of the actograms. Periodogram analysis therefore appears to be a suitable method of time series analysis by which such data may be analysed. One major problem with all methods of time series analysis, however, is the need for extensive data covering at least 10 cycles of activity, which in these studies is equal to a 10-day period. For the derived periodogram function to be an accurate representation of the data the profile of locomotor activity must be constant during the period required for analysis. The method of time series analysis can only therefore identify long term, not transient, changes in locomotor rhythmicity. Thus if antidepressant drug treatments have the ability to induce changes in the circadian rhythm of locomotor activity then such changes must be maintained throughout the period required in order for accurate identification by periodogram analysis. For this reason the methodology required that each experiment be accordingly subdivided into successive 10 day periods. Furthermore, the resolution of the periods of activity identified by periodogram analysis is dependent on the number of cycles of that period within the duration of the data analysed. Thus, in a 10 day sample (i.e. 240 hours) there are 10 cycles of 24h period length,

while there are less than 10 cycles of rhythmic activity with period lengths greater than 24h, but correspondingly more than 10 cycles of rhythmic activity with period lengths less than 24h (e.g. 20 cycles of period length 12h; 40 cycles of period length 6h). For this reason the shorter the period length the greater the resolution of the corresponding peaks in the periodogram function (see appendix B.5). Thus the accuracy of the peak in the periodogram function corresponding to the fundamental period of rhythmic activity will always be less than that of the peaks corresponding to rhythmic activity of shorter period length.

The locomotor activity monitor used to measure the activity of animals housed in groups proved to be more than adequate in that, with few exceptions, the daily median number of locomotor activity counts per epoch (i.e 15 min.) were generally greater than 20. In the individual experiments, however, the activity monitors provided comparatively low measures of locomotor activity with the daily median number of counts per epoch being invariably between 0 and 1. Periodogram analysis of data arising from grouped animals housed under L:D or D:D (constant low-intensity red light) prior to drug treatment invariably produced well-defined spectra with comparatively very low noise levels. Conversely, the noise level of the periodogram function derived from individual animals housed under L:D or D:D (constant darkness) prior to drug treatment appeared much higher. The paucity of the data arising from the individual studies probably accounts for the reduced resolution of the periodogram analysis (both in terms of the noise level and accuracy of the peaks). To increase the magnitude of the number of counts per epoch (i.e. increase the sensitivity of the monitor) it is recommended that

either the position of the single infrared cell be re-appraised or more infrared cells be provided for each monitor.

The actograms presented in these studies demonstrate the characteristic properties of true circadian rhythms. Under L:D conditions (i.e. 12h light/12h dark) the locomotor activity exhibited by both individual and grouped animals is entrained to the light-dark cycle. For the grouped animal studies at least, such observations are supported by periodogram analysis which identified the fundamental period of rhythmicity to be exactly 24h in all cases. For individual animals, however, periodogram analysis identified the fundamental period to be within the range of 23.75h. to 24.75h even though the actograms suggested an entrained 24h rhythm of locomotor activity. The inaccuracy of the derived periodogram function from individual animals is thought to be due to the paucity of data obtained in these studies. When constant conditions are imposed (i.e. constant darkness or constant low-intensity red light) the locomotor activity rhythm of both individual and grouped animals free-runs with a cycling period regulated by the intrinsic pacemakers, possible contributing environmental and, in the case of grouped animals, social factors. Both the actograms and periodogram function indicate the period of the free-running rhythm of grouped animals to be longer than that exhibited by individuals. The reason for this finding is not known. However, it is suggested that the observation may indicate the involvement of social factors in the expression of circadian locomotor activity rhythms exhibited by rats housed in groups. In a number of cases the periodogram function derived from both individual or grouped animals, housed under L:D or D:D, also indicated secondary peaks corresponding to the harmonic

components of the fundamental period. The appearance or disappearance of the harmonic components should not be assumed to indicate changes in the circadian rhythm of locomotor activity, but rather indicate changes in the shape of the wave-form of activity over 24h. During L:D conditions locomotor activity is entrained to the dark-phase. If activity occurred immediately on transition from light to dark and the level of activity remained constant throughout the dark phase, but activity stopped immediately on the transition from dark to light with no activity occurring during the light phase, then the shape of the wave-form of activity over 24h would approximate to a square-wave. Fourier analysis, on which the periodogram function is based (see Chatfield, 1985), assumes that all complex wave-forms are composed of a number of sine-waves that differ in frequency, amplitude and phase. Periodogram analysis identifies the frequency and relative amplitude of the component sine-waves within a complex wave-form. Furthermore, periodogram analysis of square-waves demonstrates that square-waves are composed of the fundamental sine-wave and the odd-numbered harmonic sine-waves. In comparison, the periodogram function of a pure sine-wave only identifies the fundamental frequency of that sine-wave (see appendix B.5 for a comparison of the periodogram functions derived from sine-waves and square-waves). The appearance or disappearance of harmonic components in the periodogram function thus indicate a change in the overall shape of the wave-form of activity. In these studies, periodogram analysis of locomotor activity exhibited by rats housed under constant conditions generally indicates a reduction in the harmonic content of the periodogram function compared to that obtained when the same animals were housed under a light-dark schedule. This indicates that following

transition from L:D to D:D the shape of the wave-form of activity over 24h changes from a wave-form which tends to a square-wave under L:D to one which tends more towards a sine-wave under D:D. The change in the shape of the wave-form of activity following changes in the lighting conditions indicates that during L:D conditions light exerts a masking effect on both the leading and trailing edges of locomotor activity expressed during the dark phase. The masking, as opposed to entraining, effects of light have been discussed by Aschoff (1988). The masking effect of light during L:D is also indicated in the actograms. The duration of activity during L:D is generally limited to the duration of the dark phase (i.e. 12h). However, during D:D the duration of the activity period gradually increases with, generally, the end of activity occurring at a slightly later time point than would be expected from the time of activity onset (e.g. see Fig 9.3).

Under constant conditions the locomotor activity rhythms of control (i.e. non-drugged) animals, housed either individually or in groups, continued to free-run for at least 40 days with little or no variation in either the resulting actograms or derived periodogram function. In all cases where examined, the free-running rhythm of control animals, housed either singly or in groups, re-entrained within 2-3 days following the introduction of L:D or D:L conditions (see Figs. 9.1, 9.3, 9.5, 9.7, 9.9, 9.11 and 9.15). In addition all control animals exhibited little or no variation in the total daily number of locomotor activity counts throughout the duration of the experiments (data not shown). However, the daily water consumption of all control animals gradually reduced throughout the experiments to approximately 80% of the starting volume by the end of

the experiment (data not shown). Periodogram analysis showed that chronic treatment with mianserin had no effect on the circadian rhythm of locomotor activity expressed by either individual or grouped animals when compared to the relevant non-drug treated animals. Indeed it was only after mianserin treatment that a disruption of the free-running rhythm was observed. Conversely, in comparison to the relevant controls, chronic administration of clomipramine or fluoxetine to either individual or grouped animals resulted in a disruption of the circadian rhythm of locomotor activity during the second or third 10-day period of drug treatment respectively, as indicated by the increased magnitude of peaks especially between 3 and 8 hours. Indeed in one experiment fluoxetine treatment of grouped rats resulted in rhythm breakdown to ultradian components with a primary period of 8.25h. No evidence of drug-induced phase shift was observed following any of the drug treatments. In all cases of rhythmic disruption, either during or, in the case of mianserin, following drug treatment the circadian rhythm of locomotor activity re-entrained within 2-3 days following the introduction of L:D or D:L conditions. The daily total of locomotor activity counts for all drug treated animals showed little or no variation throughout the course of each experiment (data not shown). However, as with the control animals, the volume of fluoxetine or mianserin drug solutions consumed gradually reduced throughout the duration of each experiment. Conversely, the fluid intake of animals presented with clomipramine was immediately reduced by up to 20% but returned to the expected level imbibed by the control animals at the same time point of the experiment following the cessation of drug treatment (data not shown). It may be argued that the reduced fluid intake of animals treated with clomipramine

may have contributed to the observed disruption of the circadian rhythm of locomotor activity, however, animals treated with fluoxetine also demonstrated rhythm disruption without reduced fluid intake. In these experiments therefore, the rhythm disruption observed during clomipramine or fluoxetine treatment would appear to be a drug effect rather than as a consequence of reduced fluid intake. In addition, such drug-induced changes in the circadian rhythm of locomotor activity may be demonstrated in both isolated and grouped animals. It should be noted, however, that the final pattern of locomotor activity exhibited by grouped animals is a composite of the individual expression of locomotor activity. In these experiments groups of 3 rats were used and it is not unreasonable to assume some slight variation between their fundamental free-running periods, or indeed in the relative phase-position of each animal's circadian rhythm of locomotor activity, even though the dominant group member may be expected to dictate the free-running rhythm of the group. Thus 3 slightly different rhythms contribute to the final pattern of activity. In this context the apparent disruption of the circadian rhythm of locomotor activity induced by clomipramine or fluoxetine in grouped animals may be due to a general increased phase separation of the individual rhythms. Thus the results for the effects of clomipramine and fluoxetine on the circadian rhythmicity of grouped animals *per se* should be treated with caution even though they are in agreement with those obtained from isolated animals.

The doses of the drugs used in these studies were based, as far as possible, on clinical equivalence. The observations that clomipramine and fluoxetine both induced disruption of the circadian

rhythm of locomotor activity of both isolated and grouped rats are generally in agreement with the effect of chronic imipramine or zimelidine on the free-running circadian locomotor activity of hamsters (Wirz-Justice and Campbell, 1982) or rats (Martin, 1982) respectively. Conversely, the lack of effect of mianserin, together with the observation that none of the drug treatments induced a phase-delay in the onset of the activity phase of the subjects sleep-wake cycle, would not support the hypothesis that an ability to alter circadian rhythms of locomotor actvity is shared by the group of compounds clinically labelled antidepressant. Likewise, Mitchell et al. (1987) observed that repeated electroshock had no effect on the circadian rhythm of locomotor activity exhibited by rats or mice. An ability to modify circadian rhythms of locomotor activity would not therefore appear to be a predictor or necessary characteristic of antidepressant efficacy.

The major criticism of the experiments described here is that they have no theoretical basis with respect to the circadian rhythm abnormalities observed in some depressed patients. In these experiments the phase-position of the circadian rhythm of locomotor activity was in its normal position with respect to other circadian rhythms, while in some depressed patients some circadian rhythms are thought to have an abnormal phase-angle to other circadian rhythms (see Wehr and Wirz-Justice, 1982). If antidepressant efficacy is indeed related to an ability to re-align abnormally positioned circadian rhythms then the animal model employed in these studies is not suitable to examine such drug-induced effects. If animal models of circadian rhythms are to be employed to identify the underlying mechanisms by which antidepressants modify circadian rhythms, or

indeed in the identification and development of potential antidepressants, then a suitable animal model needs to be developed which has a closer theoretical relationship to the circadian rhythm abnormalities observed in some depressed patients. Until such an animal model is developed it is unlikely that any direct relationship between antidepressant efficacy and an ability of antidepressant treatments to modify circadian rhythms will be identified.

The implications of the results of these studies, together with those obtained from the examination of the effect of chronic antidepressant treatment on rodent social behaviour, and suggestions for further work are discussed in chapter 10.

CHAPTER 10 GENERAL DISCUSSION

CHAPTER 10 GENERAL DISCUSSION

The results presented in this thesis have been extensively discussed in each of the relevant chapters. The purpose of this final discussion is to provide a general comment on both the methods employed and the results obtained during these studies and, furthermore, to suggest areas where further investigation would be of particular benefit.

10.1 Rodent Social Behaviour

The main bulk of this thesis has been concerned with the effect of antidepressant treatment on rodent social behaviour. These studies (see chapters 5-7) demonstrated that the effect of psychotropic drug treatment per se on patterns of both rodent social behaviour and rodent social hierarchy may be quantifiably examined. The results of these studies have been extensively discussed in chapter 8. Since the antidepressants were the only class of psychotropic compound which specifically modified aggressive behaviour it is concluded that the specific, diametrically different, effects of acute and chronic antidepressant treatment on rodent aggressive behaviour are indicative of a specific reduction and increase in social drive respectively. These results are especially impressive considering six major points. Firstly, a common effect of antidepressant treatment has been demonstrated even though the drugs studied differ markedly in chemical structure and known acute pharmacology. Secondly, a common effect was observed following either acute treatment, where only the antidepressants specifically reduced rodent aggressive behaviour at non-sedative doses, or chronic treatment, where only the antidepressants increased rodent aggressive behaviour. It is generally accepted that acute treatment

with antidepressants reduce rodent aggressive behaviour (see chapter 8). These observations also support the argument of File and Tucker (1986) who noted that the literature indicates increased aggressive behaviour to be a common behavioural effect following chronic, but not acute, antidepressant treatment of rats. Thirdly, the order of potency of the antidepressants identified by the studies described here generally correspond to that in the clinic. Fourthly, the antidepressant-induced increase in rodent aggressive behaviour was only observed following chronic treatment which is consistent with the clinical latency of these compounds (see Oswald et al., 1972). Fifthly, the time required for the drug-induced increase in aggressive behaviour observed during chronic treatment with the irreversible MAOI, phenelzine, to return to the observed pre-treatment level following the cessation of drug treatment, i.e. by 14 rather than 7 days as observed following chronic treatment with the other antidepressants, suggests that the return to the pre-treatment aggression level is due to the recovery of central monoamine oxidase activity. Lastly, the ability of acute and chronic antidepressant treatment to decrease and increase rodent aggressive behaviour, respectively, is paralleled by clinical observations where an initial reduction in mood is often observed (Oswald et al., 1972), but may be overlooked due to the inadequacies of the rating system used, or misinterpreted and categorised as a side-effect (see chapter 8), while continuous antidepressant therapy results in increased outwardly-directed (extrapunitive) aggression (Priest et al., 1980) which is manifest as increased physical and/or verbal activity associated with greater interaction with the environment (Kaplan et al., 1961). The latter clinical observations are indicative of increased social drive on the

part of the patient which, furthermore, is an integral component of remission from depression.

The ability of acute antidepressant treatment to reduce rodent aggressive behaviour is presumably mediated by the ability of these compounds to increase central serotonergic and/or noradrenergic function, even though the acute mode of action for iprindole remains to be elucidated (see section 1.4). This view is supported by the literature which indicates that increasing central serotonergic or noradrenergic function generally reduces rodent aggressive behaviour even though various types of rodent aggressive behaviour have been identified (see Moyer, 1968), indicating that various neuronal substrates may mediate such behaviour (see chapter 8). Furthermore, the literature also indicates that reducing central serotonergic or noradrenergic function increases rodent aggressive behaviour, presumably including that induced by chronic antidepressant treatment reported here (see chapter 8). These observations have a major implication for the aetiology of depression. If the close correspondence between the ability of antidepressants to modify the social drive of both laboratory rodents and depressed patients is accepted, then the studies reported here imply that remission from depression is associated with reduced central serotonergic and/or noradrenergic function which, in turn, suggests that depression may be a consequence of increased central serotonergic and/or noradrenergic function. Indeed, the literature suggests possible mechanisms by which central serotonergic and noradrenergic function may be reduced; thereby, possibly, mediating the ability of chronic antidepressant treatment to increase rodent aggressive behaviour. Firstly, post-synaptic 5-HT_2 receptors are

down-regulated following chronic antidepressant treatment (see section 1.4.5). Secondly, chronic antidepressant treatment also induces a down-regulation of central post-synaptic beta-adrenoceptors and/or a subsensitivity of the NA-dependent adenylate cyclase system (see section 1.4.5); effects possibly associated with a desensitization of the pre-synaptic $alpha_2$-adrenoceptor mediated negative feedback system.

It is generally concluded, therefore, that the antidepressant-induced modification of rodent social drive corresponds to the behavioural changes exhibited by depressed patients during the time-course of antidepressant treatment. It follows that examination of the acute and chronic effects of antidepressants in the social interaction test described in this report will predict antidepressant efficacy and drug potency. Thus the results reported here auger well for the clinical prospects of the potential antidepressant, fluoxetine. It should be noted that the ability of the social interaction test to predict antidepressant efficacy does not rely on an ability to modify, firstly, abnormal animal behaviours or biochemical deficits assumed to be consistent with a single facet of the pathology of depression or, secondly, largely abnormal behaviours induced by exotic experimental paradigms which generally have a questionable theoretical relationship to depression. The social interaction test described here has obvious potential utility for both basic academic research and the industrial screening of potential antidepressant compounds.

These views may appear somewhat premature considering the limited number of antidepressants examined thus far. Future studies should

concentrate, at least initially, on examining the effects of acute and chronic administration of clinically-established antidepressant treatments (including electroconvulsive shock), in comparison to other psychotropic compounds with no known antidepressant efficacy, on the behavioural profile exhibited by rats during social interaction; since only by the accumulation of data will the hypothesis that increased aggressive behaviour is a common behavioural effect induced by chronic antidepressant treatment be supported (or refuted !). Once the ability of this animal model to predict antidepressant efficacy is accepted then alternative, less common, antidepressant treatments may be examined. The D_2-DA antagonist flupenthixol, $beta_2$-adrenoceptor agonists such as salbutamol, and GABA agonists have all been reported to possess antidepressant efficacy (see Iversen and Mackay, 1979; Mindham, 1979; Lloyd et al., 1983). Examination of the acute and chronic treatment effects of these compounds on rodent social behaviour may confirm the general ability of this animal model to predict antidepressant efficacy regardless of the acute pharmacology of the compound being examined. Furthermore, this animal model may prove extremely useful in predicting the potential antidepressant efficacy of novel compounds.

In addition to the obvious predictive value regarding various antidepressant treatments, this animal model may also prove extremely useful in identifying the central mechanisms underlying antidepressant efficacy. Initially, the pharmacology of the antidepressant-induced modification of aggressive behaviour may be examined. The reduction in aggressive behaviour induced by acute antidepressant treatment is presumably mediated by increased

serotonergic or noradrenergic function. The use of various pharmacological agents, such as specific 5-HT and NA antagonists administered at maximal doses which do not modify the behavioural profile of rats per se, should enable the monoamine system(s) which mediate the antidepressant-induced reduction in rodent aggressive behaviour to be identified. Conversely, if reduced serotonergic or noradrenergic function mediates the increase in rodent aggressive behaviour observed during chronic antidepressant treatment, then acute treatment with agents which increase central serotonergic or noradrenergic function (i.e. with specific 5-HT or NA agonists) would be expected to reverse the antidepressant-induced increase in rodent aggressive behaviour. Assuming that central serotonergic neurons are intimately involved in the control of aggressive behaviour (see chapter 8) then the introduction of specific pharmacological agents for the ever-increasing number of 5-HT receptor subtypes should help elucidate which 5-HT receptor subtypes are involved in the mediation of increased aggressive behaviour during chronic antidepressant treatment.

Central serotonergic and noradrenergic neurons within the hypothalamic/amygdaloid/septal axis appear to mediate control over the ultimate expression of aggressive behaviour (see chapter 8). Direct application of pharmacological agents with specific actions on monoamine systems into these sub-cortical brain areas should help elucidate which monoamine systems and brain areas are intimately involved in mediating the behavioural effects of acute and chronic antidepressant treatment. Likewise, similar information may be obtained following measurement of, firstly, monoamine and metabolite levels by, for example, high performance liquid

chromatography with electrochemical detection (see Marsden and Joseph, 1986); secondly, in vivo levels of extracellular 5-HT and NA by, for example, intracerebral microdialysis (see Routledge and Marsden, 1987; Sleight et al., 1988) or voltammetry (see Crespi et al., 1988; Marsden et al., 1988); and, lastly, central 5-HT- and NA-sensitive adenylate cyclase activity and phosphatidylinositol turnover in these brain areas. Such biochemical studies should be performed, in conjunction with behavioural testing, prior to, during and following antidepressant treatment as applicable. A number of studies have demonstrated that chronic antidepressant treatments induce a down-regulation of specific monoamine receptors (i.e. alpha$_2$- and beta-adrenoceptors, 5-HT$_2$ receptors) and a subsensitivity of the NA-sensitive adenylate cyclase system (see section 1.4.5 for discussion). Generally, such studies have understandably employed brain tissue that either has a high density of the receptor subtype or is rich in the second messenger system being studied. For example, the cerebral cortex has commonly been employed to study the radioligand-binding characteristics of alpha$_1$- and alpha$_2$-adrenoceptors, beta-adrenoceptors, 5-HT$_1$ and 5-HT$_2$ receptors (see, for example, Savage et al., 1979; Peroutka and Snyder, 1980; Savage et al., 1980a, 1980b; Sellinger-Barnette et al., 1980; Blackshear and Sanders-Bush, 1982; Clements-Jewery and Robson, 1982; Mogilnicka et al., 1987; Sethy et al., 1983), while the limbic forebrain (which contains the septal nuclei, olfactory tubercules, olfactory nuclei, accumbens nuclei and anterior parts of the anterior amygdaloid nuclei) has commonly been employed to study the NA-dependent adenylate cyclase system (see, for example, Vetulani et al., 1976a; Mishra et al., 1981). If the primary target of antidepressant drug action is shown to be the

hypothalamic/amygdaloid/septal axis, then it is clear that more relevant data regarding the mechanisms involved in the efficacy of antidepressants would be obtained if the suggested pharmacological and biochemical studies outlined above concentrated on examining the effect of antidepressant treatment on monoamine function in these sub-cortical brain areas.

10.2 Circadian Rhythms of Locomotor Activity

A far smaller proportion of this thesis has been concerned with the effect of chronic antidepressant treatment on the circadian rhythms of locomotor activity in rats and the applicability of time-series analysis to the resulting data (see chapter 9). The results of these studies suggest that while periodogram analysis may be employed to identify changes in the rhythmic components of circadian locomotor activity (which may not always be obviously apparent in the resulting actogram), an ability to modify the free-running circadian locomotor activity rhythms of rodents is neither a predictor nor necessary characteristic of antidepressant efficacy. This conclusion was reached considering, firstly, the difference between the latency of clomipramine and fluoxetine to induce disruption of the free-running circadian rhythms of locomotor activity; secondly, the inability of clomipramine and fluoxetine to induce a phase-shift in the free-running circadian locomotor activity rhythm; and, lastly, the total lack of effect of mianserin. It should be noted that as far as possible the three compounds studies were administered at clinically-equivalent doses. If the antidepressant efficacy of these compounds is related to an ability to modify circadian rhythms, then it is reasonable to expect a degree of commonality between the effects of the three compounds in this animal model. This expectation

assumes that the use of the animal model employed in these studies is justified which, as previously discussed (section 9.4), is not the case. Furthermore, each daily dose of the compounds used in these studies was far greater than that which induced an increase in the aggressive behaviour of resident rats during social interaction. This suggests that one major problem with the experimental design used in these studies may possibly be the presentation of the drug in the drinking water since in this case the subject chooses when to drink (or not !) - thereby exercising complete control over the timing of drug administration - and, furthermore, it must be assumed that the drug becomes widely distributed throughout the CNS. Perhaps a better method would be to administer the drug directly to the central pacemaker systems (e.g. the SCN) via indwelling cannulae linked to automatic drug delivery systems (e.g. subcutaneously implanted osmotic mini pumps). Such a method of drug delivery would allow the operator to decide to administer the drug either continuously or in pulses. A second major problem lies in the assumption that the underlying mechanisms generating the normal free-running circadian rhythms of locomotor activity are susceptible to antidepressant drug action. It has been suggested by some workers (e.g. see Wehr and Wirz-Justice, 1982) that the phase-position of certain circadian rhythms are advanced in relation to other circadian rhythms in some depressed patients. The desynchronization between circadian rhythms is then manifest in the depressive symptamology. The clinical observations therefore indicate that it is the phase-relationship between circadian rhythms which is important rather than an individual circadian rhythm per se. The experimental design used in these studies would be greatly improved by the additional, simultaneous, monitoring of feeding and drinking behaviour,

temperature and, for grouped rats, social behaviour. Thus the effect of antidepressant treatment on the phase-relationship between various free-running circadian rhythms may be examined. Such an experimental paradigm may not be particularly relevant if the various circadian rhythms maintain their relative phase-position to each other, even when allowed to free-run under constant environmental conditions. If one or more of the circadian rhythms may be either disrupted or abnormally phase-positioned (perhaps by periodic food or water deprivation; see Stephan, 1984; Clarke and Coleman, 1986; Stephan, 1986a, 1986b, 1986c) then, it is argued, the animal model would have a greater degree of relevance to the circadian rhythm abnormalities observed in some depressed patients.

10.3 Conclusions

The studies on rodent social behaviour have clearly demonstrated that the ability of chronic antidepressant treatment to increase social drive in rodents may be a behavioural affect common to the chemically-disparate group of compounds labelled antidepressant. It is further argued that the rodent social interaction model employed in these behavioural studies predicts antidepressant efficacy and drug potency without relying on either an interaction with pharmacological tools or exotic experimental paradigms. Conversely, it is also concluded that an ability to modify the free-running circadian locomotor activity rhythms of rodents is neither a predictor nor necessary characteristic of antidepressant efficacy. The social interaction model described in this thesis provides a method by which antidepressant efficacy may be examined which may lead to a greater understanding of the mechanisms involved in the clinical efficacy of antidepressant treatment.

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APPENDICES

APPENDICES

Appendix A Capture of Rodent Social Interaction Behaviours.

A.1 Description

Monitoring rodent social behaviour is necessarily both intense and time consuming. There are over 40 various elements of rodent behaviour as described by Silverman (1965) which may be grouped into motivational categories. Silverman identified 4 'residual' behaviours (i.e. those with no identifiable motivational category) which occur very rarely and these have been excluded from the analysis. The various elements of behaviour may change very quickly and hence it is impossible for the observer to record the behaviour of both animals concurrently. To overcome this problem the period of social interaction was recorded on to video tape for analysis at a later date, thus enabling the observer to record each animals behaviour in turn. The program, written in BASIC for use with a BBC microcomputer, was written specifically to aid recording of rodent social activity. Each of the behavioural elements identified by Silverman may be input into the computer by means of a 2 letter abbreviation (Table A.1). The program continuously compares the input abbreviation against a check-list of acceptable abbreviations, provided in the DATA statements, and ignores any unacceptable input. The abbreviations may be altered according to the requirements of the experimenter. Once accepted the abbreviation is stored in the computers memory together with the time of observation. The program also monitors the duration of the recording period to ensure a constant monitoring period between animals and experiments. The program has been designed to accept the sequential behaviours of one animal at a time and is then automatically re-run in order to monitor the second animal's behaviour. At the end of each experiment the

data is stored on disk for retrieval at a later date.

A listing of the program is provided in section A.2.

Category	Behaviour	Abbreviation
i) Exploration	Explore	EX
	Rear	RE
ii) Investigation	Approach	AA
	Nose and investigate	IG
	Follow	FL
	Sniff (genitalia)	SS
	Mount	MT
	Attempt mount	AM
	To-fro	TF
	Walk round / circle / side	WR
	Stretched attention	SA
	Tail rattle	RA
iii) Grooming and Consummatory	Wash	WG
	Lick	LG
	Scratch	SG
	Shake	SK
	Lick penis	LP
	Dig	DI
	Eat	EC
	Drink	DC
iv) Aggression	Aggressive posture	AP
	Aggressive groom	AG
	Bite	BT
	Pull	PL
	Threat and thrust	TT
	Attack	AK
	Offensive upright	OU
	Offensive sideways	OS
v) Flight-Submit	Defensive upright	DU
	Defensive sideways	DS
	Submit	SU
vi) Flight-Escape	Retreat	RT
	Flag/Evade	FE
	Crouch	CC
	Elevated crouch	EV
	Under food hopper	UN
	Attend	AT

Table A.1 Rodent Social Interaction Behaviours.

A.2 Listing

```
 10REM Started 20-Nov-85
 20REM LAST UP-DATE 01-01-86
 25REM ECONET VERSION 15-MAY-86( 04-04-87)
 30REM C.R.I.B. : Program to Capture Rodent Interaction Behaviours.
 80CLS
 90PRINT"This program is designed to capture"
100PRINT"data on Rodent Interaction Behaviours"
110PRINT"obtained from the social interaction"'"test and recorded
   on to video tape."
113PRINT'"The program is designed to accept"'"behavioural
   abbreviations as they occur"'"and is automatically re-run to
   collect"'"data for the second animal in the group."
120PRINT"P.J.Mitchell November 1985"
130PRINT"Bath University"
140PRINT''''"Press <space-bar> to continue.";
150REPEAT
160G=GET
170UNTIL G=32
180CLS
190Q=0
200REPEAT
210PRINT"This program accepts new data input via"'"the keyboard or
   retrieves data"'"previously stored on disc by
   this"'"program."''"New data is automatically stored"'"on disc
   and printed out."
220PRINT''"****************** MENU ******************"
230PRINT'TAB(12)"N - New Data"
240PRINTTAB(12)"R - Retrieve Data"
250PRINTTAB(12)"E - Exit Program"
260PRINT''"****************************************"
270G=GET
280IF G=78 THEN PROCnew:GOTO200
290IF G=82 THEN PROCretrieve:GOTO200
300IF G=69 THEN END
310IF G<>78 AND G<>82 AND G<>69 GOTO270
320UNTIL G=0
330REM ****************************
340REM PROC NEW
350REM ****************************
360DEF PROCnew
370Q=Q+1
380PROCintro
390PROCbehaviour
400IF Q=1 THEN PROCdefine
410IF Q>1 THEN PROCdimclear
420INPUT'"Date (dd-mm-yy) ",date$
500INPUT'"Animal Code ";animal$
502INPUT'"Dose/Drug/Route ";treat$
504INPUT'"Pre-treatment time ",pretime$
506INPUT'"Partner animal Code ";panimal$
520PROCdatacollect
550PROCsave
560CLS
570ENDPROC
```

```
 580REM ****************************
 590REM PROC RETRIEVE
 600REM ****************************
 610DEF PROCretrieve
 630PROCdefine
 650CLS
 660INPUT"Type in full file specification"'"(including drive and
    directory if required) "''file$
 670X=OPENIN file$
 680INPUT£X,date$,animal$,panimal$,treat$,pretime$,endepoch,M
 690FOR I=1 TO (2*B)
 700INPUT£X,D$(I)
 710NEXT I
 720FOR I=1 TO M
 730INPUT£X,L(I),B$(I)
 740NEXT I
 760CLOSE£X
 780ENDPROC
 790REM ****************************
 800REM PROC DEFINE
 810REM ****************************
 820DEF PROCdefine
 830I=0:B=0
 840REPEAT:READ B$:B=B+1:UNTIL B$="zzzz":B=B-1
 850RESTORE
 900DIM D$(2*B),FQ(2*B),L(1250),B$(1250)
 903FOR I=1 TO 2
 905A=0
 910REPEAT:READ B$:A=A+1
 920IF I=1 THEN D$(A)=STR$(I)+" "+B$
 925IF I=2 THEN D$(A+B)=STR$(I)+" "+B$
 930UNTIL A=B
 940RESTORE
 950NEXT I
 960RESTORE
1310ENDPROC
1320REM ****************************
1330REM PROC DATA COLLECT
1340REM ****************************
1350DEF PROCdatacollect
1360TS=0:TE=0:T1=0
1370REM TS = TIME START : ON <S>
1380REM TE = TIME EPOCH END : ON <CR> OR 10 MINS MAX
1390REM T1 = TIME BEHAVIOUR START : ON BEHAVIOUR ABBREVIATION
1420REM LATENCY = T1-TS
1430M=0
1445FOR R=1 TO 2
1447IF R=2 PRINT"Rewind tape to start of epoch "'"to analyse second
    animals behaviour."
1450PRINT'"Press S to start the clock. "
1460REPEAT
1470G=GET
1480IF G<>83 PRINT'"I said press S to start the clock."
1490UNTIL G=83
1500TS=TIME
1510PRINT'"Clock Running."
1640REPEAT
```

```
 1650Z=0
 1655REPEAT
 1660G$=GET$
 1665G=ASC(G$):IF G=13 THEN TE=TIME:
     endepoch=(TE-TS)/100:PRINT'"Length of epoch= "endepoch"
     secs.":GOTO1910
 1670Z=Z+1
 1680IF Z=1 THEN T1=TIME:first$=G$
 1690IF Z=2 THEN second$=G$
 1700UNTIL Z=2
 1710total$=STR$(R)+" "+first$+second$
 1720PRINT total$;
 1725 I=0:C=0
 1730REPEAT I=I+1
 1735IF I>2*B THEN PRINT,"Behaviour NOT KNOWN !":PRINT CHR$(7):
     GOTO 1745
 1740C=INSTR(total$,D$(I))
 1745UNTIL C=1 OR I>2*B
 1750IF I>2*B AND C=0 THEN first$=second$:Z=1:GOTO 1655
 1820M=M+1:B$(M)=total$
 1822PRINT,M,(T1-TS)/100'
 1824L(M)=(T1-TS)/100
 1837T=TIME
 1840IF ((T-TS)/100)>600 THEN endepoch=(T-TS)/100:PRINT'"Lenght of
     epoch= "endepoch" secs.":GOTO 1910
 1850UNTIL M>1249
 1900CLS
 1910NEXT R
 1920INPUT'"Enter file name for data storage "file$
 1930ENDPROC
 2580REM ****************************
 2590REM PROC PRINT
 2600REM ****************************
 2610DEF PROCprint
 2620VDU2
 2630 PRINT TAB(15)"Capture of Rodent Interaction Behaviours."
 2634 PRINT TAB(15)"======================================="
 2638PRINT''TAB(15);"Date      ";date$;TAB(40);"File      ";file$
 2640PRINT''TAB(15);"Test Animal       ";animal$;TAB(40);"Partner
Animal     ";panimal$
 2642PRINTTAB(15);"Treatment          ";treat$
 2644PRINTTAB(15);"Treatment Time     ";pretime$
 2660PRINT TAB(15)"Length of Epoch    ";endepoch;" secs"'
 2662M=0:I=0:N=0:C=0
 2664REPEAT M=M+1:UNTIL L(M)=0:M=M-1
 2666FOR I=1 TO (2*B)
 2668IF I=1 THEN PRINT TAB(9);"Test animal "animal$
 2670IF I=B+1 THEN PRINT'TAB(9);"Partner animal "panimal$
 2672@%=&05
 2673IF I=1 OR I=(B+1) THEN PRINT
TAB(8);"------------------------------------------------------------"
 2674IF I=1 OR I=(B+1) THEN PRINT
TAB(10)"N";TAB(16)"D$"TAB(22)"FQ"TAB(34)"Latencies"
 2675IF I=1 OR I=(B+1) THEN PRINT
TAB(8);"------------------------------------------------------------"
 2676PRINT TAB(6)I;TAB(15);D$(I);
 2678FOR N=1 TO M
```

```
 2680C=INSTR(D$(I),B$(N))
 2685IF C=1 THEN FQ(I)=FQ(I)+1
 2690C=0
 2695NEXT N
 2700PRINT TAB(19)FQ(I);
 2705IF FQ(I)=0 THEN PRINT TAB(34)"Zero Observations."
 2710IF FQ(I)>0 THEN PROClatencies(I,M)
 2715IF I=B THEN PRINT
TAB(8);"------------------------------------------------------------"
 2720NEXT I
 2723PRINT
TAB(8);"------------------------------------------------------------"
 2725PRINT TAB(9)"Total number of observations    = "M
 2726FOR X=1 TO B:Y=Y+FQ(X):NEXT X:PRINT TAB(9)"Observations for Test
     animal    = "Y:Y=0
 2728FOR X=(B+1) TO (2*B):Y=Y+FQ(X):NEXT X:PRINT TAB(9)"Observations
     for Partner animal = "Y:Y=0
 2730PRINT
TAB(8);"------------------------------------------------------------"
 2733VDU12:VDU3
 2735ENDPROC
 2740REM ******************************
 2745REM PROC LATENCIES
 2750REM ******************************
 2755DEF PROClatencies(I,M)
 2760@%=&20206
 2763Z=0
 2765FOR N=1 TO M
 2770C=INSTR(D$(I),B$(N))
 2775IF C=1 THEN Z=Z+1
 2780IF Z>5 THEN Z=1
 2785IF C=1 THEN PRINT TAB(20+(Z*8)) L(N);
 2790C=0
 2795NEXT N
 2800@%=&A
 2810PRINT
 2820ENDPROC
 2830REM ******************************
 2840REM PROC SAVE
 2850REM ******************************
 2860DEF PROCsave
 2880PRINT'"Data to be stored""under file ";file$
 2890INPUT'"Do you wish to change""the file specification ";Y$
 2900IF Y$="Y" THEN INPUT'"Type in the new file specification :
     "'file$
 2910X=OPENOUT file$
 2920PRINT£X,date$,animal$,panimal$,treat$,pretime$,endepoch,M
 2930FOR I=1 TO (2*B)
 2940PRINT£X,D$(I)
 2950NEXT I
 2960FOR I=1 TO M
 2970PRINT£X,L(I),B$(I)
 2980NEXT I
 3000CLOSE£X
 3010ENDPROC
```

```
3020REM ****************************
3030REM PROC DIM CLEAR
3040REM ****************************
3050DEF PROCdimclear
3060FOR I=1 TO (2*B)
3070FQ(I)=0
3080NEXT I
3090FOR I=1 TO M
3100L(I)=0:B$(I)="zz"
3110NEXT I
3150ENDPROC
3410REM ****************************
3420REM PROC INTRO
3430REM ****************************
3440DEF PROCintro
3450CLS
3460PRINT'"Type in the behaviour abreviation"'"each time that
    behaviour is observed."
3540PRINT'"Press <CR> to indicate epoch end."''''''''''
3550PRINT'"Press <space-bar> to continue.";
3560REPEAT
3570G=GET
3580UNTIL G=32
3590CLS
3600ENDPROC
3610REM ****************************
3620REM PROC BEHAVIOUR
3630REM ****************************
3640DEF PROCbehaviour
3650PRINT TAB(10)"Behavioural Activity."
3660PRINT TAB(10)"----------- ---------"
3670PRINT'"1) Exploration   2) Investigation"
3675PRINT"                    and Mating"
3680PRINT'"EX  EXplore       AA  AproAch"
3690PRINT"RE  REar          IG  nose and"'"
    InvestiGate"
3700PRINT"3) Maintenance    FL  FoLlow"
3710PRINT"                  SS  Sniff"
3720PRINT"WG  Wash Groom    MT  MounT"
3730PRINT"LG  Lick Groom    AM  Attempt Mount"
3740PRINT"SG  Scratch       TF  To-Fro"
3750PRINT"SK  ShaKe            <Walk Round"
3760PRINT"LP  Lick Penis    WR <circle"
3770PRINT"DI  DIg              <side"
3780PRINT"EC  Eat           SA  Stretched AttentionDC  Drink
    RA  tail RAttle"
3820PRINT''"Press <space-bar> to continue.";
3830REPEAT:G=GET:UNTIL G=32:CLS
3835PRINT"4) Aggression     5) Flight-Escape"'
3837PRINT"AP  Aggressive    RT  ReTreat"
3840PRINT"    Posture       FE  Flag/Evade"
3842PRINT"AG  Aggressive    CC  CrouCh"
3844PRINT"    Groom         EV  EleVated crouch"
3845PRINT"BT  BiTe          UN  UNder hopper"
3847PRINT"PL  PuLl          AT  ATtend"
3850PRINT"TT  Threat and"
3852PRINT"    Thrust        6) Flight-Submit"
```

```
3854PRINT"AK  AttacK"
3856PRINT"OU  Offensive    DU  Defensive"
3858PRINT"    Upright          Upright"
3860PRINT"OS  Offensive    DS  Defensive"
3862PRINT"    Sideways         Sideways"
3864PRINT"                   SU  SUbmit"
3866PRINT"7) Residual"'
3868PRINT"TU  ToUch"
3870PRINT"CH  CHange position"
3872PRINT"RU  RUb"
3874PRINT"BC  BounCe"
3875PRINT''"Press <space-bar> to continue.";
3876REPEAT:G=GET:UNTIL G=32:CLS
3878ENDPROC
3880REM ****************************
3890REM DATA
3900REM ****************************
3910DATA EX,RE,AA,IG,FL,SS,MT,AM,TF,WR
3914DATA SA,RA,AP,AG,BT,PL,TT,AK,OU,OS
3918DATA DU,DS,SU,RT,FE,CC,EV,UN,AT,WG
3920DATA LG,SG,SK,LP,DI,EC,DC,zzzz
3930REM ****************************
```

Appendix B Time Series Analysis of Circadian Rhythms of Locomotor Activity

B.1 Introduction

A time series is a collection of observations made sequentially in time, and methods of analysing time series constitute an important area of statistics. In this report time series analysis has been applied to the circadian locomotor activity of rats in order to identify the periodicity of the behaviour (although the basic principles equally apply to any area where observations indicate a periodic change of a parameter over time). For background reading on the subject of time series analysis and its application to chronobiology the reader is directed to Chatfield (1985) and De Prins et al. (1986), respectively. For the purpose of this report the application of time series analysis to rodent circadian locomotor activity will be limited to a discussion of the applicability of the calculated periodogram and autocovariance functions. Initially both methods of time series analysis will be described, followed by a discussion on the relative merits of both methods when applied to the circadian rhythms of rodent locomotor activity.

B.2 Periodogram analysis.

The periodogram was invented in 1898 by Sir Arthur Schuster. He was fitting a model with one frequency, f, of the form

$$x_t = A\cos 2\pi ft + B\sin 2\pi ft + e_t$$

to the set of points x_0, x_1, x_2, $x_3 \ldots x_{T-1}$, where A and B are constants and e_t is a set of zero mean, variance σ^2, normally

distributed independent errors.

The least-squares estimates of A and B are

$$\hat{A} = 2/T\sum_{t=0}^{T-1} x_t \cos 2\pi ft$$

$$\hat{B} = 2/T\sum_{t=0}^{T-1} x_t \sin 2\pi ft$$

If we take the periodic function (i.e. without e_t), and take it's sum of squares using the estimates for A and B, then we obtain

$$\sum_{t=0}^{T-1} (\hat{A}\cos 2\pi ft + \hat{B}\sin 2\pi ft)^2$$

This quantity will be proportional to the total energy of the series at the frequency f.

Dividing the quantity by 4π gives the definition of the periodogram, denoted by I(f). Thus,

$$I(f) = 1/2\pi T \left\{ (\sum_{t=0}^{T-1} x_t \cos 2ft)^2 + (\sum_{t=0}^{T-1} x_t \sin 2ft)^2 \right\}$$

This produces a set of A's and B's for each frequency in the harmonic series estimated by A_j and B_j, and thus at each frequency,

$$I(f_j) = T/4\pi \left[(A_j)^2/2 + (B_j)^2/2 \right]$$

Thus the total variability ($T\sigma^2$) accounted for by each frequency is proportional to the squares of the amplitude at that frequency.

The equation

$$I(t) = 1/N\pi \left\{ \sum_{t=0}^{N} x_t \cos(2\pi\ t/T) \right\}^2 + \left\{ \sum_{t=0}^{N} x_t \sin(2\pi\ t/T) \right\}^2$$

where t = 1, 2, 3...N

x_t = observation at time t

T = period of interest,

extends the periodogram to enable any period to be analysed rather than just relying on the harmonic series.

B.3 Autocovariance Function Analysis.

B.3.1 Autocorrelation Coefficients

An important guide into the properties of a time series is provided by a series of quantities called sample autocorrelation coefficients. These measure the correlation between observations at different distances (k) apart and is given by the equation,

$$r_k = \sum_{t=1}^{N-k}(x_t - \bar{x})(x_{t+k} - \bar{x}) \Big/ \sum_{t=1}^{N-k}(x_t - \bar{x})^2$$

r_k is called the autocorrelation coefficient at lag k.

In practice the autocorrelation coefficients are usually calculated by computing the series of autocovariance coefficients, C_k, which are defined as,

$$C_k = 1/N \sum_{t=1}^{N-k}(x_t - \bar{x})(x_{t+k} - \bar{x})$$

i.e. the autocovariance coefficient at lag k.

Plotting C_k against k produces the variogram.

From the values of C_k we may derive the autocorrelation coefficients r_k, where $r_k = C_k/C_o$

Plotting r_k against k produces the correlogram.

B.3.2 Relationship between the periodogram and the autocovariance function.

The periodogram is defined as

$$I(t) = 1/N\pi\left\{\sum_{t=1}^{N}x_t\cos(2\pi t/T)\right\}^2 + \left\{\sum_{t=1}^{N}x_t\sin(2\pi t/T)\right\}^2$$

also $\cos(2\pi t/T) = \cos(2\pi pt/N)$

and $\sin(2\pi t/T) = \sin(2\pi pt/N)$

thus $2\pi t/T = 2\pi pt/N$

and $1/T = p/N$

The periodogram may therefore be written as

$$I(w_p) = \left\{\sum_{t=1}^{N} x_t \cos(2\pi\, pt/N)\right\}^2 + \left\{\sum_{t=1}^{N} x_t \sin(2\pi\, pt/N)\right\}^2$$

however, $w_p = 2\pi\, p/N$ for integer values of p, where p/N = the number of cycles per time interval.

Thus,

$$I(w_p) = 1/N\pi\left\{\sum_{t=1}^{N}(x_t - \bar{x})\cos w_p t\right\}^2 + \left\{\sum_{t=1}^{N}(x_t - \bar{x})\sin w_p t\right\}^2$$

$$= 1/N\pi\left\{\sum_{t=1}^{N}(x_t - \bar{x})(x_s - \bar{x})(\cos w_p t \cos w_p s + \sin w_p t \sin w_p s)\right\}$$

Since $C_k = \sum_{t=1}^{N-k}(x_t - \bar{x})(x_{t+k} - \bar{x})/N$

and $\cos w_p k = \cos w_p t \cos w_p (t+k) + \sin w_p t \sin w_p (t+k)$

then by substitution,

$$I(w_p) = (C_o + 2\sum_{k=1}^{N-1} C_k \cos w_p k)/\pi$$

i.e. the autocovariance function at w_p

When the periodogram is expressed in this form it appears as an obvious estimate of the power spectrum where

$$f(w) = (\gamma_o + 2\sum_{k=1}^{\infty} \gamma_k \cos wk)/\pi$$

Here γ_o and γ_k are replaced by their estimates C_o and C_k respectively for values of k up to (N-1).

Thus the periodogram is the discrete Fourier transform of the complete sample autocovariance function. However the precision of C_k decreases as k increases and so it is reasonable to give less weight to the values of C_k as k increases.

An estimator which has this property is

$$f(w) = (\lambda_o C_o + 2\lambda_k C_k \cos wk)/\pi$$

where λ_o and λ_k are a set of weights called lag windows and M (which is <N) is called the truncation point.

In comparing the summary equations for $I(w_p)$ and f(w) it can be seen that the values of C_k for M<k<N are no longer used, while values of C_k for k<M are weighted by a factor of λ_k

A lag window which may be used is the Tukey window which acts as a low pass filter and is defined as

$$\lambda_k = (1+\cos k/M)/2$$

where k = 0,1,2,....M

and λ_o is where k = 0 (i.e. $\lambda_o = 1$)

B.4 Application of periodogram and autocovariance function analysis to circadian rhythms of locomotor activity

The autocovariance function assumes that the data is normally distributed (i.e. the sample mean equals the sample median). However the distribution of the data arising from the circadian locomotor activity studies, of both single and grouped animals described in this report, is highly skewed (see Figs. B.1a and B.1b respectively), i.e. the sample mean is far greater than the sample median.

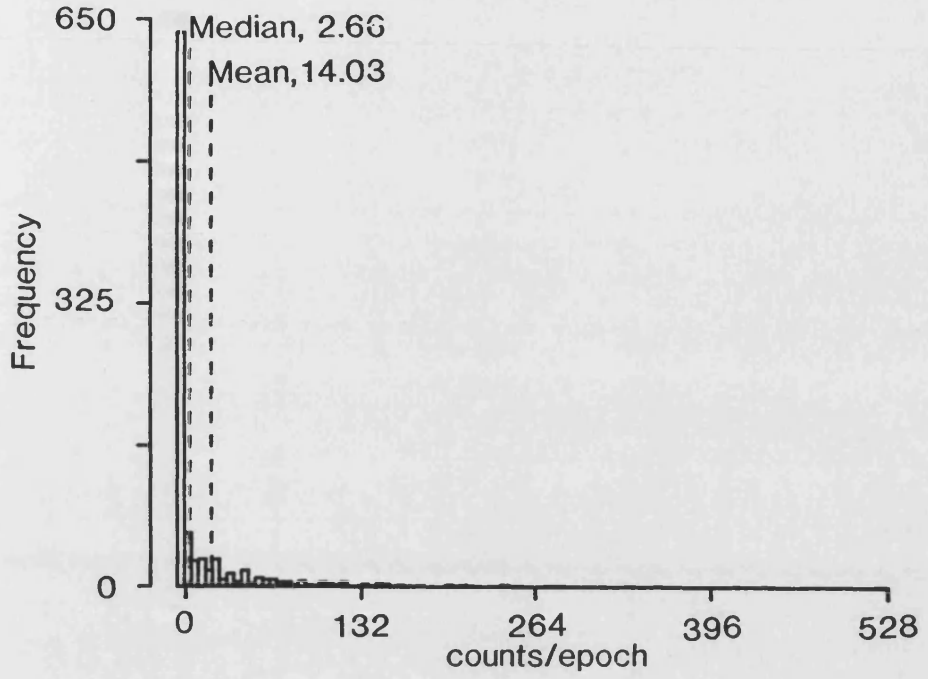


Fig. B.1a Histogram of locomotor activity counts : Single animal

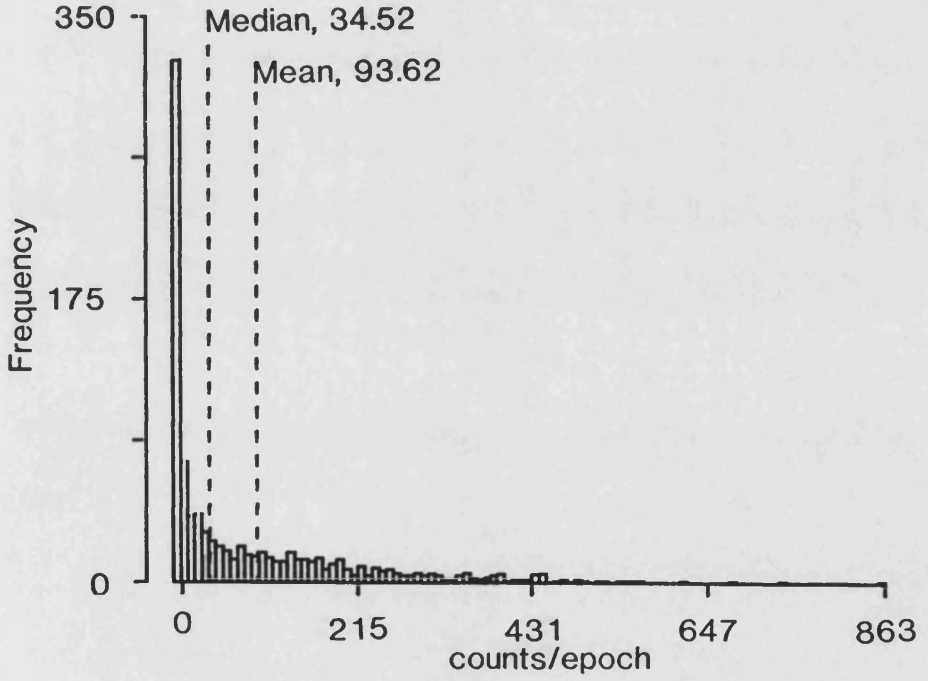


Fig. B.1b Histogram of locomotor activity counts : Grouped (N=3) animals

In order to approximate the data to a normal distribution prior to determining the autocorrelation coefficients it is therefore necessary to transform the raw data so that the sample mean tends toward the sample median. Fig. B.2 shows the mean/median ratio plotted against the root transformation factors 1 to 5 of the data used in Fig. B.1b (obtained from grouped animals housed under normal daylight. Invariably a simple cube-root transformation results in the sample mean approaching the sample median and thus satisfies the demands made by this method of analysis. Fig B.3 shows the distribution of data, obtained from grouped animals, following cube-root transformation. However, as shown in Fig. B.4, the cube-root transformation results in a drastic reduction in the magnitude of the data such that the rhythmic nature of locomotor activity is not so well defined. In the experiments where rats were individually housed the distribution of the data, in addition to the skewness, was such that invariably the sample median was less than 1 count per epoch (i.e. the majority of epochs contained zero counts, Fig B.1a). In this data sample, 494 of the 960 epochs (i.e. >50%) contained zero counts and therefore cube-root transformation to normalize the distribution of the data was not applicable. For these reasons the applicability of autocovariance function analysis to circadian locomotor activity is highly questionable. Conversely, periodogram analysis makes no assumptions about the distribution of data and thus may be applied to the raw locomotor activity data.

A listing of the program to determine the periodogram function of circadian locomotor activity, specifically for use on a BBC microcomputer in conjunction with data files produced by Marshall's program (see section 4.4.2.4) is provided in section B.6.

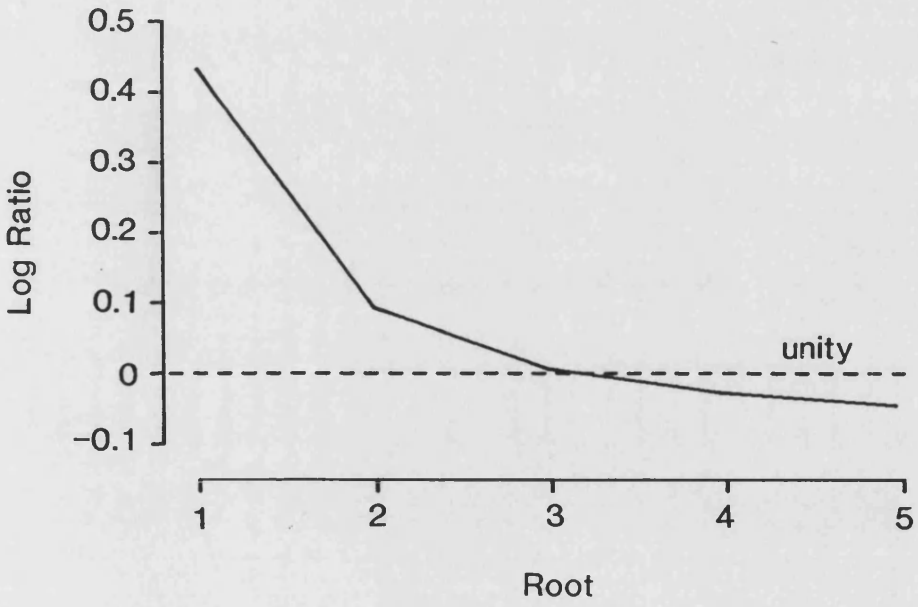


Fig. B.2 Sequential root transformation of locomotor activity data : Grouped rats

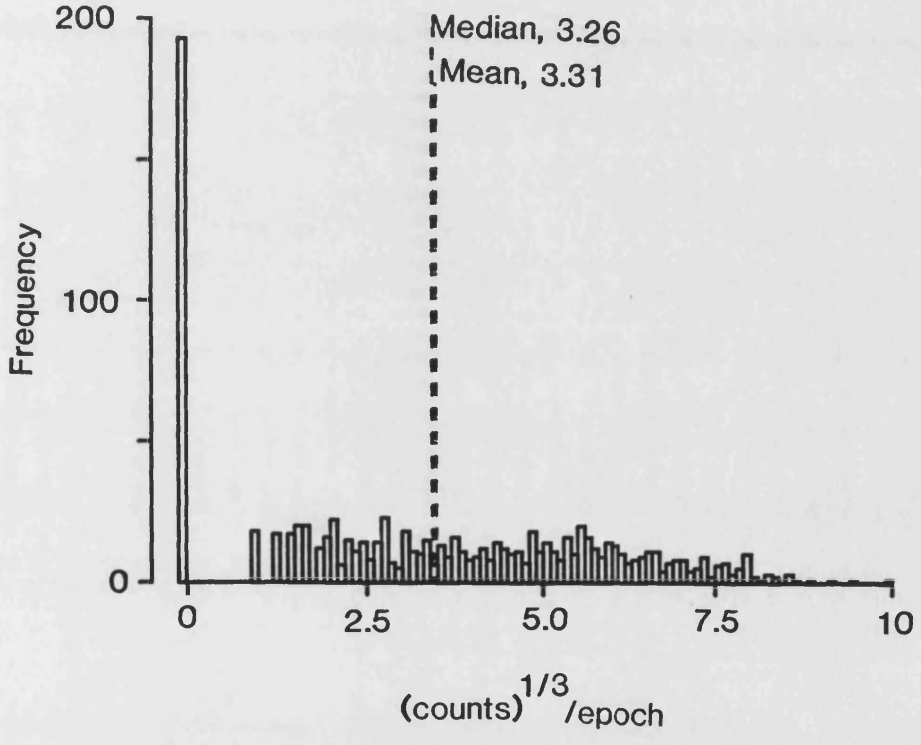


Fig. B.3 Histogram of locomotor activity following cube-root transformation : Grouped rats

Fig. B.4 Comparison between the absolute locomotor activity counts following cube-root transformation : Grouped (N=3) rats

a) Original data

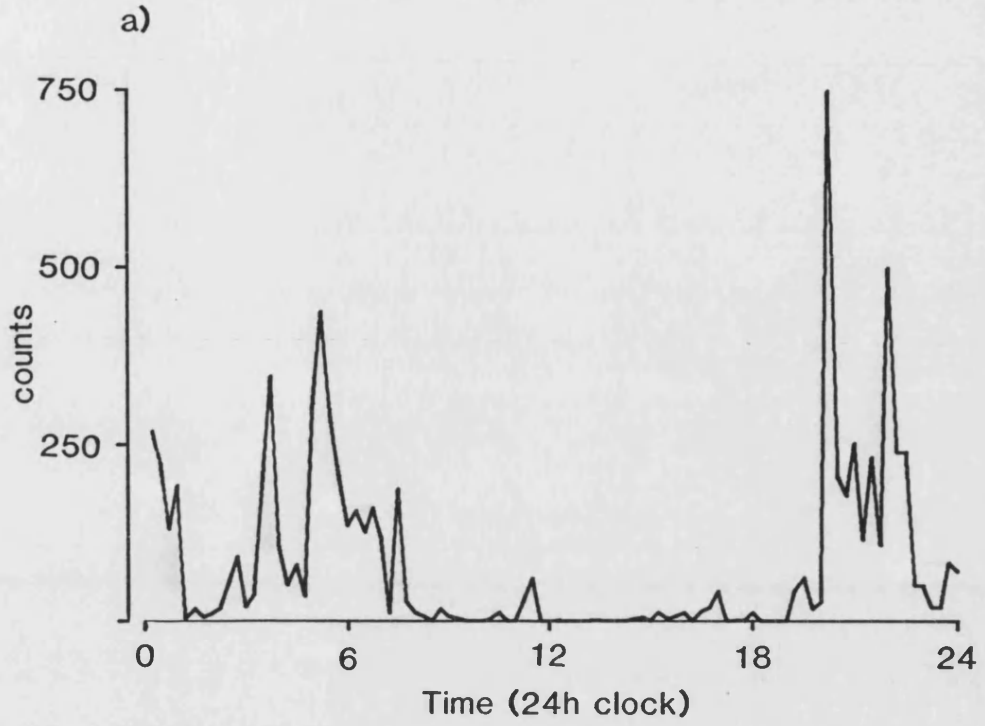
b) Cube-root transformed data

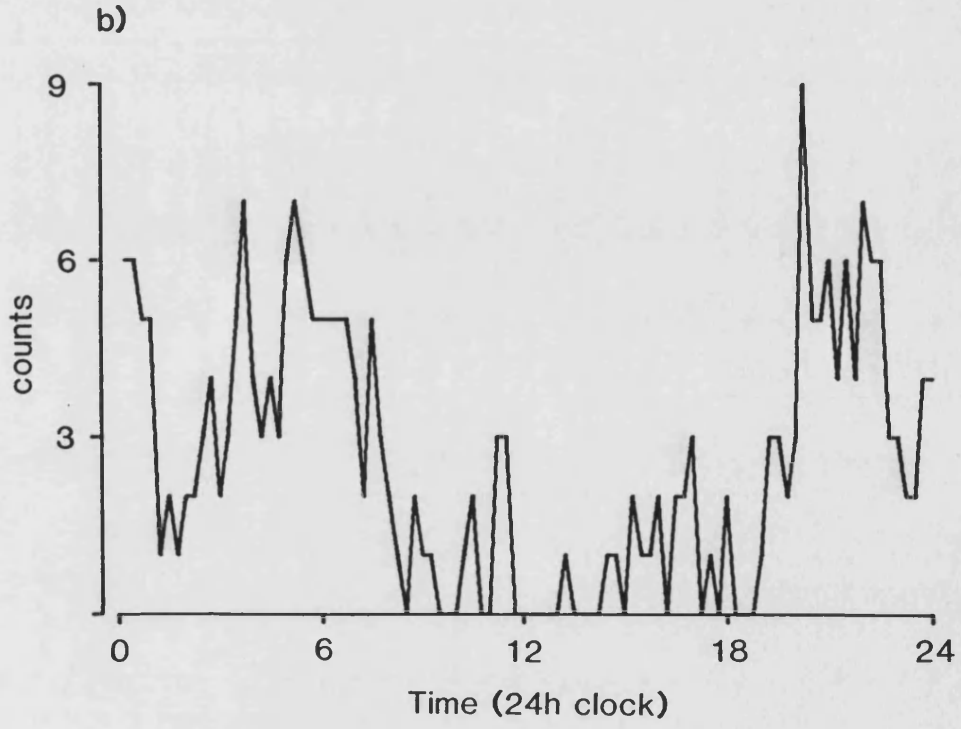
Abscissa : Time (24h clock)

Ordinate : Locomotor activity counts

Data indicates locomotor activity counts collected over 24h

Rats housed under L:D conditions (lights on 0800)


a)
750
500
250
counts
0
6
12
18
24
Time (24h clock)


b)
9
6
3
counts
0
6
12
18
24
Time (24h clock)

B.5 Practical considerations of periodogram analysis.

Adequate time series analysis inherently involves manipulation of large amounts of data. It is therefore important to determine the amount of data required by these methods in order to gain meaningfull results. Periodogram profiles were determined from 2, 5, 10 and 20 days samples of data obtained from rats housed in groups under normal daylight conditions (Fig. B.5). Analysis of just 2 days data produces very wide curves with poorly defined peaks (Fig. B.5a). Increasing the number of days to 5, slightly increases the resolution of the main peaks but also increases the level of apparent noise, especially for periods less than 8 hours, and produces new peaks, some of which may be spurious, in the same area (Fig. B.5b). Analysis of 10 days data produces well defined peaks for the fundamantal period and its harmonics (Fig. B.5c). However, there are three peaks between 18 and 22 hours, initially hidden, which appear as the resolution of the periodogram is increased. Periodogram analysis of 20 days data produces very well defined peaks with little or no apparent noise or spurious peaks (Fig. B.5d). From a practical viewpoint however, the analysis of 20 days data has three major drawbacks. Firstly, the duration of the periodogram analysis on this amount of data is approximately 4 hours (the programme was written in BASIC and is therefore rather slow, see section B.6). Secondly, a requirement of 20 days data for each stage of the experiment results in very long experiments. Thirdly, if any drug-induced effects upon circadian locomotor activity cannot be maintained for 20 days then the ability of periodogram analysis to identify significant changes in activity is reduced. It was therefore decided to accept the shortcomings of analysing over 10

Fig. B.5 Periodogram analysis : Locomotor activity expressed by grouped rats (N=3) housed under L:D.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{3}$).

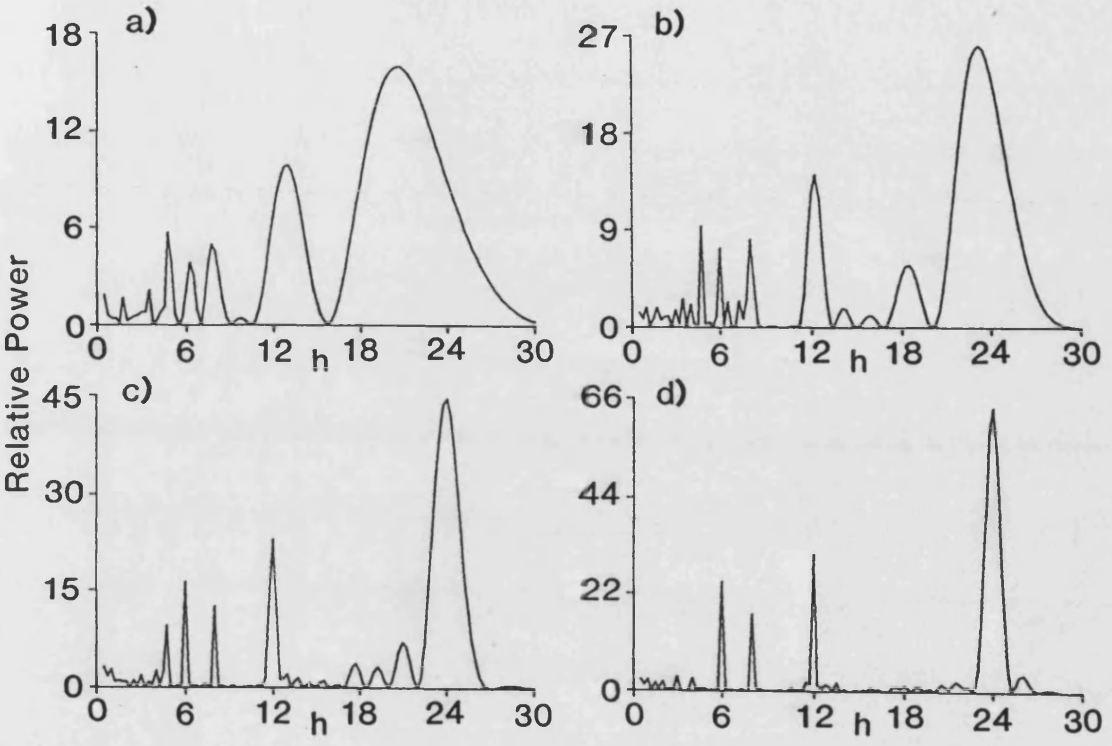
a, 2 days (192 epochs)

b, 5 days (480 epochs)

c, 10 days (960 epochs)

d, 20 days (1920 epochs)

Data collected in 15min epochs.


a)
b)
c)
d)
Relative Power
18
12
6
0
27
18
9
0
45
30
15
0
66
44
22
0
0 6 12 h 18 24 30

day periods and design the locomotor studies accordingly (see section 9.2.2.4). In order to standardize the analysis of data the periodogram function was determined on 10 day samples of data in all cases.

A major problem with periodogram function analysis is it readily identifies the harmonic components of the rhythms fundamental period, which may confuse interpretation of the derived function. The harmonic components of a waveform are those with periods that are integer multiples of the fundamental. Simple sine waves do not contain any harmonics. Examples of the derived periodogram function following analysis of sine waves with fundamental periods of 24h, 12h, 6h and 3h are shown in Fig B.6. In each case the periodogram shows a single peak indicating the period of the rhythm. By comparison, the derived periodogram function following analysis of square waves, with fundamental periods of 24h, 12h, 6h and 3h, identifies not only the fundamental period but also the harmonic components of the waveforms (Fig B.7). In each example the peaks of the periodogram function of the square waves indicate the odd harmonics of the fundamental period, i.e. 3rd, 5th, 7th, 9th...etc. The occurrence of harmonic components therefore indicate the shape of the waveform being analysed. When periodogram function analysis is applied to circadian rhythms of locomotor activity the harmonic components thus indicate the general daily wave pattern of locomotion and their generation or disappearance should not be assumed to indicate large changes in the rhythmicity of circadian activity. Conversely, relatively small peaks (compared to the fundamental) in the periodogram function that occur at periods which

Fig. B.6 Periodogram function derived from sine waves.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{3}$).

Data sample equivalent to 240h of 15min epochs (960 data points) in each case.

a, Fundamental period = 24h (No. of cycles = 10)

b, Fundamental period = 12h (No. of cycles = 20)

c, Fundamental period = 6h (No. of cycles = 40)

d, Fundamental period = 3h (No. of cycles = 80)

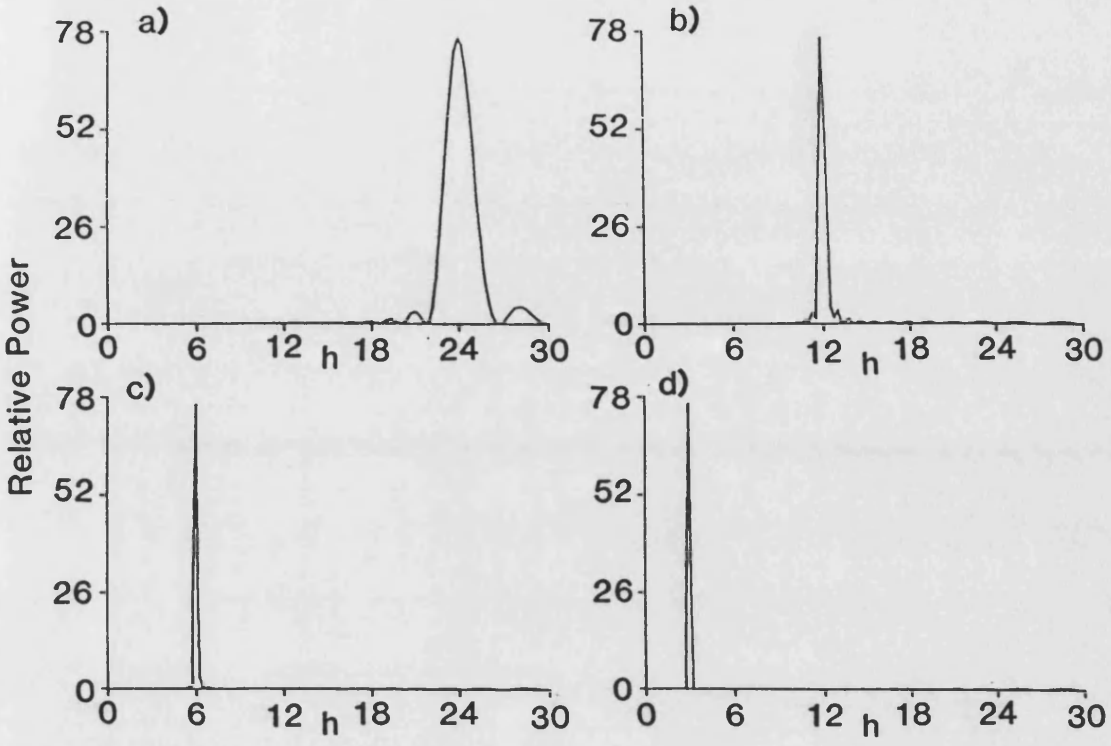

a)
b)
c)
d)
Relative Power
78
52
26
0
0
6
12
h
18
24
30

Fig. B.7 Periodogram function derived from square waves.

Abscissa: Period length (h). Ordinate: Relative power ($*10^{3}$).

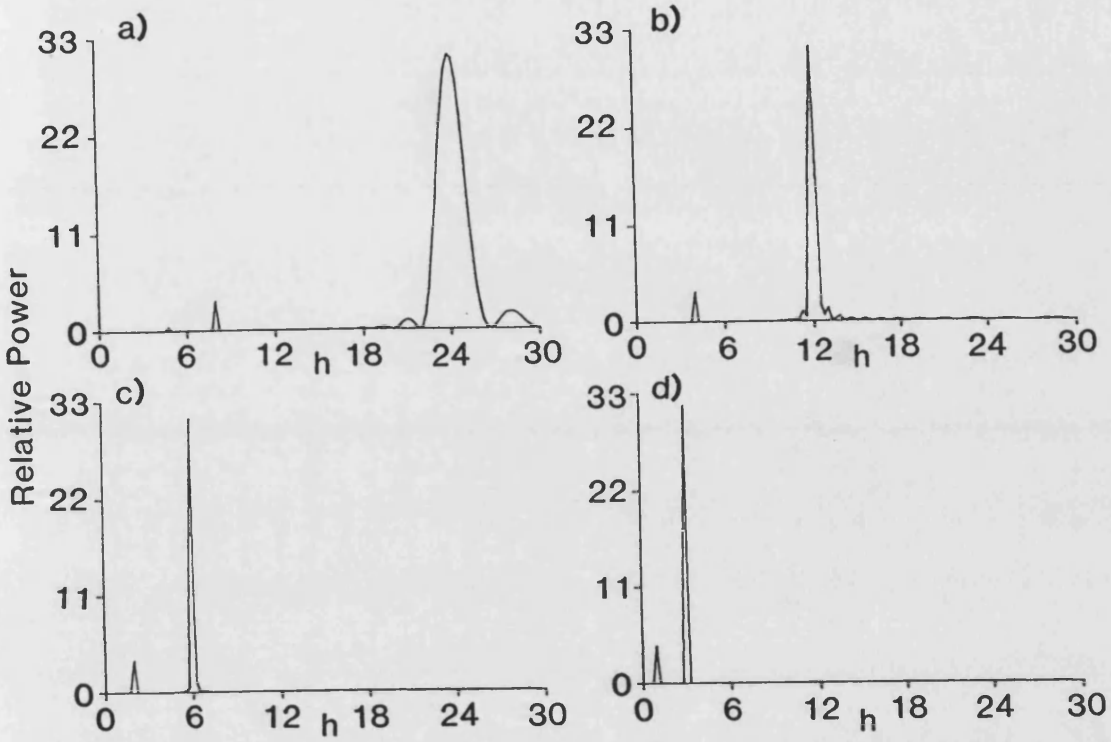
Data sample equivalent to 240h of 15min epochs (960 data points) in each case.

a, Fundamental period = 24h (No. of cycles = 10)

b, Fundamental period = 12h (No. of cycles = 20)

c, Fundamental period = 6h (No. of cycles = 40)

d, Fundamental period = 3h (No. of cycles = 80)


a)
b)
c)
d)
Relative Power
33
22
11
0
0
6
12
h
18
24
30

are not integer multiples of the fundamental period may indicate relatively large changes in the circadian rhythm of locomotor activity. The analysis of square waves indicates the basic premise of Fourier analysis, i.e. that all complex waveforms are composed of a series of sine waves that differ in their period (i.e. frequency), amplitude and relative phase position.

The periodograms shown in Figs. B.6 and B.7, following analysis of sine and square waves respectively, were each derived from the equivalent of 10 days data and indicate that the resolution of each peak is dependent upon the number of cycles of that period contained in the data sample. In addition, analysis of cosine waves with fundamental periods of 24h, 12h, 6h and 3h produces periodogram functions identical to those obtained following analysis of sine waves (data not shown). The derived periodogram function per se therefore contains no information regarding the relative phase position of the component sine waves.

Thus while the use of the periodogram function may prove useful in identifying the rhythmic components of circadian locomotor activity the interpretation of the derived periodogram function should always be treated with care.

B.6 Listing

```
10REM ROUTINE TO CALCULATE AND PLOT THE FRQUENCY SPECTRA OF RODENT
  LOCOMOTOR ACTIVITIES.  P.J. MITCHELL APRIL 1986;  UNIVERSITY OF
  BATH
20REM LAST UPDATE 14-MAY-86
25REM PERIODOGRAM ANALYSIS
30
40ON ERROR GOTO 3820
50CLEAR
60CLOSE£0
70REM ************************
80REM"******** SPECTRA ********"
90REM ************************
100CLS
110PRINT'TAB(10)CHR$(141)"ACTIVITY SPECTRA"
120PRINT TAB(10)CHR$(141)"ACTIVITY SPECTRA"
130PRINT''TAB(12)CHR$(141)"*** MENU ***"
140PRINT TAB(12)CHR$(141)"*** MENU ***"
150PRINT'STRING$(39,"*")
160PRINT''"N...Analysis of New data"
170PRINT'"R...Retrieval of spectra file from disc"
180PRINT'"E...Exit programme"
190PRINT''STRING$(39,"*")
200PRINT''"Input choice ? ";
210G$=GET$
220IF G$="N" PROCNEW
230IF G$="R" PROCRETRIEVE
235IF G$="E" END
240MODE4
250PROCgraph
260MODE7
270CLS
280PRINT TAB(0,0)"ORIGINAL DATA FILE INFORMATION."
290PROCINFOOUT
300PRINT TAB(11,12)"Spectral Analysis."
310PRINT TAB(3,14);"Days ";SDAY%;" to ";EDAY%;" completed."
320PRINT TAB(3,16)"Spectral data stored on file ";File$
330IF FNYN("Press <Y> for further analysis",22)="Y" THEN GOTO 40
340END
350
360DEF PROCNEW
370CLS
380PRINT'"THIS PROGRAM CALCULATES THE POWER"'"SPECTRA OF LOCOMOTOR
   ACTIVITY COLLECTED"'"BYLOCOAND STORED ON DISK"
390REM "GET SETUP DATA FROM DISK"
400PROCDISKINFO
410CLS
420PROCINFOOUT
430DIM MAG(30*SMN%),PT%(LCD%+1)
440PROCpointer
450PROCCL(11)
460IF NBX%>1 THEN BN%=FNR("WHICH BOX/CELL DO YOU WISH TO
   ANALYSE",11,1,NBX%) ELSE BN%=1
470PROCCL(11)
```

```
480PRINT TAB(0,11)"SEQUENTIAL ANALYSIS FROM DAY TO
   DAY":PROCsequential
490FOR DAY%=SDAY% TO EDAY%
500IF NBX%>1 PRINT TAB(5,19)"PROCESSING  BOX ";BN%
510PRINT TAB(5,21)"TRANSFERRING DAY ";DAY%
520PROCDAYGET
530NEXT DAY%
540File$=(":3.S."+STR$(BN%)+"-"+STR$(SDAY%)+"-"+STR$(EDAY%))
550PROCCL(21)
560PRINT TAB(0,21)"DATA TO BE STORED IN FILE "File$
570IF FNYN("IS THIS FILE NAME O.K.",23)="N" THEN PROCCL(23):PRINT
   TAB(0,23)"TYPE IN NEW FILE NAME ";:INPUT File$:PROCCL(23):PRINT
   TAB(0,21)"DATA TO BE STORED IN FILE "File$
580PROCspectra
590PROCprintout
600PROCsave
610ENDPROC
620
630REM ****************************
640REM"**PROC TO LOAD DISK INFO**"
650REM ****************************
660DEF PROCDISKINFO
670IF G$="N" THEN NF$=FNLT("NAME OF FILE TO PLOT",8,0,7)
680IF G$="R" THEN NF$=FNLT("NAME OF FILE TO PLOT",8,0,9)
690IF NF$<>"" AND (ASC(NF$)<65 OR ASC(NF$)>123) THEN PRINT
   TAB(0,24)CHR$(7)"FILENAMES SHOULD START WITH A LETTER";:GOTO 670
700DR$=":1."
710F%=OPENUP(DR$+NF$)
720IF F%<>0 THEN 790
730IF DR$=":1." THEN DR$=":3.": GOTO 710
740PRINT TAB(0,24)CHR$(7)TAB(0,24)"FILE NOT FOUND - TRY
   AGAIN";TAB(0,10);
750*CAT 0
760Q$=INKEY$(1000)
770*CAT 1
780GOTO 670
790INPUT£F%,DN%,TC%,AEOD%,CEOD%
800INPUT£F%,NBX%,ED%,NBR%,SD%,LCD%,SI%
810IF G$="R" GOTO 890
820PTR£F%=100
830INPUT£F%,DT$
840PTR£F%=200
850DIM DCR$(NBX%)
860FOR I%=1 TO NBX%
870INPUT£F%,DCR$(I%)
880NEXT I%
890SMN%=60/SI%
900ENDPROC
910
920DEF PROCINFOOUT
930@%=&A
940PRINT TAB(0,1)"Title:- "DT$
950PRINT'"Disk NO. ";DN%
960PRINT'"NO. of Boxes/Cells in use ";NBX%
970PRINT'"Start Day ";SD%"           End Day ";LCD%+1
980PRINT'"Data Accumulation Period ";SI%" Min"
990ENDPROC
```

```
1000
1010DEF PROCpointer
1020PRINT TAB(0,11)"OBTAINING POINTER VALUES - PLEASE WAIT"
1030PTR£F%=1000
1040PT%(1)=1000
1045PDAY%=SD%
1050FOR I=(SD%+1) TO (LCD%+1)
1060PTR£F%=PT%(I-1) + (NBR%*(60/SI%)*24)
1100REPEAT
1110PTR£F%=PTR£F%-NBR%
1120PT%=PTR£F%
1130TESTD%=BGET£F%
1140PTR£F%=PT%
1150UNTIL TESTD%=PDAY%
1160PT%=PT%+NBR%
1162PTR£F%=PT%
1164PDAY%=BGET£F%:PHR%=BGET£F%:PMIN%=BGET£F%
1165IF PDAY%>TESTD% AND PHR%=0 AND PMIN%=0 THEN PT%=PT%+NBR%
1166PTR£F%=PT%
1170PT%(PDAY%)=PT%
1175I=PDAY%
1180NEXT I
1190ENDPROC
1200
1210DEF PROCsequential
1220PROCCL(13)
1230SDAY%=FNR("START AT DAY",13,1,LCD%+1)
1240EDAY%=FNR("END AT DAY",15,SDAY%,LCD%+1)
1250NDAYS%=(EDAY%-SDAY%)+1
1260PRINT TAB(0,17)"SEQUENTIAL ANALYSIS OF ";NDAYS%;" DAYS."
1270DIM SAMPLE%(NDAYS%*24*SMN%)
1280ENDPROC
1290
1300REM*****************************
1310REM"**PROC TO GET 1DAYS DATA**"
1320REM*****************************
1330DEF PROCDAYGET
1340BOFF%=(BN%-1)*2
1350PTR£F%=PT%(DAY%)
1360REPEAT
1370PT%=PTR£F%
1380TDAY%=BGET£F%:IF TDAY%=0 GOTO 1470
1390HR%=BGET£F%
1400MN%=BGET£F%
1410IF HR%=0 AND TDAY%=DAY%+1 THEN HR%=24
1420MN%=MN%/SI%
1430I%=(HR%*SMN%+MN%)
1440PTR£F%=PTR£F%+BOFF%
1450SAMPLE%(96*(DAY%-SDAY%)+I%)=BGET£F%+256*BGET£F%
1460PTR£F%=PT%+NBR%
1470UNTIL TDAY%>DAY% OR TDAY%=0
1480ENDPROC
1490
1500DEF PROCspectra
1510P2=2*3.14159
1520PROCCL(19)
1530FOR I%=2 TO 30*SMN%
```

```
1540@%=&2020A
1550PRINT TAB(0,23)"PROCESSING TIME PERIOD ";I%/SMN%
1560A=0:B=0
1570FOR t%=1 TO NDAYS%*24*SMN%
1580A=A+(SAMPLE%(t%)*COS(P2*t%/I%))
1590B=B+(SAMPLE%(t%)*SIN(P2*t%/I%))
1600NEXT t%
1610MAG(I%)=(A^2+B^2)/(NDAYS%*24*SMN%*3.14159)
1620NEXT I%
1630@%=&A
1640ENDPROC
1650
1660DEF PROCprintout
1670*FX3,10
1680PRINT TAB(15)"SPECTRAL ANALYSIS OF LOCOMOTOR ACTIVITY DATA."
1690PRINT TAB(15) STRING$(45,"="):PRINT'
1700PRINT"Original data file information."
1710PRINT STRING$(31,"-"):PRINT
1720PROCINFOOUT
1730PRINT'
1740PRINT TAB(25)"*** SPECTRAL ANALYSIS. ***"'
1750PRINT TAB(25)"Box/Cell NO.        ";BN%
1760PRINT TAB(25)"Analysis Start Day ";SDAY%
1770PRINT TAB(25)"Analysis   End Day ";EDAY%
1780PRINT TAB(25)"Maximum Period     30 Hrs."
1790PRINT TAB(25)"SPECTRAL DATA FILE ";File$
1800PRINT'STRING$(79,"-")
1810PRINT TAB(31)"Spectral Analysis."
1820PRINT TAB(2)"Period"TAB(15)"Time"TAB(28)"Power"
    TAB(45)"Period"TAB(58)"Time"TAB(71)"Power"
1830PRINT STRING$(79,"-")
1840column%=(30*SMN%)/2
1850FOR I%=2 TO column%+1
1860@%=&6:PRINT TAB(0)I%;
1870@%=&2020A:PRINT TAB(9)I%/SMN% TAB(24)MAG(I%);
1880IF I%=column%+1 GOTO 1910
1890@%=&6:PRINT TAB(43) I%+column%;
1900@%=&2020A:PRINT TAB(52) (I%+column%)/SMN% TAB(67)
    MAG(I%+column%)
1910NEXT I%
1920PRINT'STRING$(79,"-")
1930PRINT
1940IF G$="R" GOTO 2050
1950Total=0:sd=0
1960FOR I%=1 TO NDAYS%*24*SMN%
1970Total=Total+SAMPLE%(I%)
1980NEXT I%
1990MEAN=Total/(NDAYS%*24*SMN%)
2000FOR I%=1 TO NDAYS%*24*SMN%
2010sd=sd+ABS(SAMPLE%(I%)-MEAN)
2020NEXT I%
2030sd=sd/(NDAYS%*24*SMN%-1)
2040crit=(sd*5.99/P2)
2050PRINT "Mean= "MEAN
2060PRINT "S.D.= "sd
2070PRINT "95% = "crit
2080PRINT STRING$(79,"-")
```

```
2090*FX3,0
2100ENDPROC
2110
2120DEF PROCsave
2130X%=OPENOUT(File$)
2140PRINT£X%,DN%,TC%,AEOD%,CEOD%
2150PRINT£X%,NBX%,ED%,NBR%,SD%,LCD%,SI%
2160PRINT£X%,BN%,SDAY%,EDAY%,DT$
2170FOR I%=2 TO 30*SMN%
2180PRINT£X%,MAG(I%)
2190NEXT I%
2200PRINT£X%,MEAN,sd,crit
2210CLOSE£X%
2220ENDPROC
2230
2240DEF PROCRETRIEVE
2250CLS
2260PROCDISKINFO
2270INPUT£F%,BN%,SDAY%,EDAY%,DT$
2280DIM MAG(30*SMN%),PT%(LCD%+1)
2290FOR I%=2 TO 30*SMN%
2300INPUT£F%,MAG(I%)
2310NEXT I%
2320INPUT£F%,MEAN,sd,crit
2330CLOSE£0
2340File$=NF$
2350IF FNYN("Print out data ",10)="Y" THEN PROCCL(10):PROCprintout
2360ENDPROC
2370
2380DEF PROCgraph
2390VDU24,0;0;1279;1023;
2400VDU28,0,4,39,0
2410CLS:CLG
2420PROCxaxis
2430PROCyaxis
2440PROCmax
2450X=107:XOFF=1140/(30*SMN%):Y=0:YOFF=93
2460PROCyscale
2470PROCplot
2480ENDPROC
2490
2500DEF PROCxaxis
2510MOVE 107,93:DRAW 1247,93
2520FOR I=0 TO 5
2530MOVE 107+(I*228),93
2540DRAW 107+(I*228),77
2550NEXT I
2560VDU5
2570MOVE 91,70:PRINT"0"
2580MOVE 319,70:PRINT"6"
2590MOVE 531,70:PRINT"12"
2600MOVE 759,70:PRINT"18"
2610MOVE 987,70:PRINT"24"
2620MOVE 1215,70:PRINT"30"
2630MOVE 469,32:PRINT"Period  (Hrs)"
2640VDU4
2650ENDPROC
```

```
2660
2670DEF PROCyaxis
2680MOVE 107,93:DRAW 107,843
2690FOR I=0 TO 3
2700MOVE 107,93+(I*250)
2710DRAW 91,93+(I*250)
2720NEXT I
2730ENDPROC
2740
2750DEF PROCmax
2760max%=0
2770FOR I=2 TO 30*SMN%
2780IF max%<MAG(I) THEN max%=MAG(I)
2790NEXT I
2800ENDPROC
2810
2820DEF PROCplot
2830X=X+(2*XOFF)
2840YCAL=750/yaxis%
2850MOVE X,YCAL*MAG(2)+YOFF
2860FOR I%=3 TO SMN%*30
2870X=X+XOFF
2880Y=YOFF+YCAL*MAG(I%)
2890DRAW X,Y
2900NEXT I%
2910PROCheader
2920ENDPROC
2930
2940DEF PROCyscale
2950PRINT TAB(0,0)"Maximum counts per epoch = ";max%
2960INPUT TAB(0,1)"Input max. y value ";yaxis%
2970I=1
2980REPEAT I=I+1
2990scale%=yaxis%/10^I
3000UNTIL scale%<10
3010scale%=10^I
3020VDU5
3030@%=&2010A
3040MOVE 55,109:PRINT"0"
3050fact=(yaxis%/scale%)
3060MOVE 0,359:PRINT;fact/3
3070MOVE 0,609:PRINT;2*fact/3
3080MOVE 0,859:PRINT;fact
3090MOVE 32,544:PRINT"P"
3100MOVE 32,513:PRINT"0"
3110MOVE 32,481:PRINT"W"
3120MOVE 32,449:PRINT"E"
3130MOVE 32,417:PRINT"R"
3140@%=&A
3150MOVE 0,246:PRINT"10";:MOVE 64,262:PRINT;I;
3160VDU4
3170@%=&A
3180ENDPROC
3190
3200DEF PROCheader
3210FOR I%=0 TO 4
3220PROCCL(I%)
```

```
3230NEXT I%
3240INPUT TAB(0,0)"Treatment  ?"treat$
3250INPUT TAB(0,1)"Conditions ?"cond$
3260PROCCL(0):PROCCL(1)
3270PRINT TAB(0,2)"Day ";SDAY%;" to "EDAY% TAB(18,2)"Data File ";
    File$
3280PRINT TAB(0,3)"Treatment.."treat$
3290PRINT TAB(0,4)"Conditions."cond$
3300INPUT TAB(0,0)"DO YOU REQUIRE A PRINT-OUT ";DUMP$;
3310PROCCL(0)
3320PRINT TAB(11,0)"SPECTRAL ANALYSIS";
3330IF DUMP$="Y" OR DUMP$="y" THEN PROCDUMP
3340ENDPROC
3350
3360REM*************************
3370REM**"PROC TO HANDLE I/O"**
3380REM*************************
3390DEF FNR(M$,L,B,T)
3400LOCAL II%,FG%,LL%,N$
3410PRINT TAB(0,L)M$" 3420INPUT"";N$
3430PRINT TAB(0,24)STRING$(39," ");
3440FG%=0
3450FOR II%=1 TO LEN(N$)
3460IF ASC(MID$(N$,II%,1))=46 THEN 3480
3470IF ASC(MID$(N$,II%,1))<48 OR ASC(MID$(N$,II%,1))>57 FG%=1
3480NEXT II%
3490IF FG%=1 THEN PRINT TAB(0,24)CHR$(7)"REDO NON NUMERIC CHARACTER
    DETECTED";:GOTO 3410
3500IF VAL(N$)>=B AND VAL(N$)<=T THEN =VAL(N$)
3510PRINTTAB(0,24)CHR$(7)"RANGE- MUST LIE BETWEEN ";B;" & ";T;:
    GOTO 3410
3520ENDPROC
3530
3540DEF FNYN(M$,L)
3550LOCAL Q$
3560PRINT TAB(0,L)M$" 3570INPUT"";Q$
3580PRINT TAB(0,24)STRING$(39," ");
3590IF Q$="Y" OR Q$="y" THEN ="Y"
3600IF Q$="N" OR Q$="n" THEN ="N"
3610PRINT TAB(0,24)CHR$(7)"ANSWER MUST BE Y FOR YES OR N FOR NO";
3620GOTO 3560
3630ENDPROC
3640
3650DEFFNLT(M$,L,B,T)
3660LOCAL Q$
3670PRINT TAB(0,L)M$" 3680INPUT""Q$
3690PRINT TAB(0,24)STRING$(39," ");
3700IF LEN(Q$)=0 AND B>0 THEN PRINT TAB(0,24)CHR$(7)"NULL ANSWER NOT
    ACCEPTABLE";:GOTO 3670
3710IF LEN(Q$)<B OR LEN(Q$)>T THEN PRINT TAB(0,24)CHR$(7)"MUST HAVE
    BETWEEN ";B;" & ";T" LETTERS";:GOTO 3670
3720=Q$
3730ENDPROC
3740
3750DEFPROCCL(P%)
3760PRINT TAB(0,P%) STRING$(39," ")
3770ENDPROC
```

```
3780
3790DEF PROCDUMP
3800* :2.U.SDUMP
3810ENDPROC
3820REM*****************************
3830REM"***ERROR HANDLING*******"
3840REM*****************************
3850CLS
3860IF ERR=17 THEN CLOSE£0
3870PRINT TAB(0,2)"ERR=";ERR,"ERROR LINE=";ERL
3880REPORT:PRINT "IS ERROR"
3890
3900END
```

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